STUDIES ON GROWTH AND THE NUTRITIONAL, METABOLIC, AND HORMONAL STATUS OF CHILDREN WITH CHRONIC RENAL FAILURE ON REGULAR HAEMODIALYSIS.

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ABSTRACT

The purpose of this study was to assess the nutritional, metabolic and hormonal status of children with chronic renal failure, and to study the pathogenesis of the growth retardation which is an important clinical problem affecting these children. Sixteen children on regular haemodialysis were observed over a period of one year.

Growth was subnormal in most patients with delay in skeletal maturation, and growth potential fell with advancing bone age. Body weight was less affected than height presumably because all children received energy supplements in their diet. Puberty was delayed but pubertal children grew better and had a more normal body cell mass than prepubertal children. Significant increases were found in the fasting levels of blood glucose, plasma insulin, growth hormone, glucagon, cortisol, luteinizing hormone, prolactin, triglycerides, cholesterol, glycine and alanine, and significant decreases were observed in plasma thyroxine, triiodothyronine, testosterone, branched-chain amino acids, free fatty acids and glycerol. Growth velocity correlated positively with plasma valine, leucine and gonadotrophin levels. In boys, growth velocity correlated positively with plasma testosterone and inversely with prolactin levels. Plasma triglyceride concentration was related to both carbohydrate intake and plasma insulin level. There were also positive correlations between growth hormone and fatty acids, growth hormone and the branched-chain amino acids, serum somatomedin and plasma transferrin, and between transferrin and the % deficit in weight for height and for age. Extracellular water was increased and intracellular water (cell mass) was decreased, but body fat was

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normal or increased in most patients. Glucose intolerance with hyperinsulinaemia, non-suppressable hyperglucagonaemia and paradoxical rises in plasma growth hormone levels after a glucose load were observed. Fasting plasma alanine concentration correlated inversely with glucose disappearance rate (Kg). The fall in plasma free fatty acids was more marked than normal and was related to plasma insulin response. Basal oxygen consumption was increased particularly in the more wasted children. Manipulation of the diet for a further period of one year by increasing the ratio of polyunsaturated to saturated fats lowered plasma cholesterol level but not plasma triglycerides.

On the basis of the findings, it is suggested that the primary defect is a peripheral resistance to insulin with respect to glucose utilization accompanied by a decrease in the mobilization of endogenous fat for energy production and a diversion of amino acids for energy purposes with an increase in hepatic gluconeogenesis and body protein depletion. It was also suggested that energy and protein requirements of uraemic children may be increased, and that energy supplements as carbohydrate would increase body fat without affecting cell mass.

It is hoped that the information obtained from the study will be helpful in further understanding the pathogenesis of growth retardation in children with chronic renal failure and their nutritional needs, and will provide guidelines for future research.

DECLARATION

This thesis contains a number of new observations, and deductions have been made on many of the topics examined. I have personally designed the study, performed all the clinical work described in the thesis and carried out many of the hormone assays and oxygen consumption measurements. Others who kindly performed analytical measurements for me are duly acknowledged.

Though I have held many discussions with others, who are acknowledged, all of the conclusions and hypothesis embodied in this thesis are my own.

I declare that this thesis is composed by me and is my own work.

Parts of the study have been published and a bibliography and reprints are included in Appendix (D).

M.M. El Beshty.

PREFACE

The Clinical Problem and Aims of the Study

A variety of metabolic and endocrine disturbances are known to occur in adults with chronic renal failure. While many of the biochemical changes associated with the uraemic state are reversed or ameliorated by haemodialysis, some persist despite adequate treatment.

It is now clearly established that haemodialysis can, technically, be applied to children as successfully as to adults, and an increasing number of children with end-stage renal failure are being treated by long term regular haemodialysis both in hospital and in the home in preparation for renal transplantation. The survival rate is probably better in this age group than similarly treated adult patients. However, poor growth and development affecting children with chronic renal failure both before and after the institution of regular haemodialysis continues to be a major problem in dialysis and transplantation programmes as it can have a tremendous psychological effect on the child interfering with the quality of his life. Between 1972 and 1976, 40% of the children accepted for haemodialysis and renal transplantation at Guy's Hospital, London were below the third percentile for height. Although a number of factors such as renal osteodystrophy and calorie deficiency are known to contribute, the pathogenesis of this disorder remains obscure.

Of the various factors that are known to affect growth, nutritional, hormonal and metabolic factors play a dominant role. Information regarding the effects of chronic renal failure and dialysis on these determinants of growth have been fragmentary and in most instances derived from studies on either adult patients or experimental animals.

Studies on uraemic children are scanty and often a single parameter was measured in a group of patients whereas the need is to study all parameters in the same child so as to assess inter-relationships.

In view of the complexity of growth mechanisms, it is difficult to approach the problem from one aspect only. This thesis, therefore, does not aim to define one specific abnormality by detailed study of a confined area, but is a general view of the nutritional, hormonal and metabolic status of uraemic children on haemodialysis in order to (1) shed light on the possible mechanisms underlying the abnormal growth in chronic renal failure, (2) to further our understanding of the needs of these children particularly in respect of their nutritional care, and (3) to define specific areas for future research.

Historical perspectives and/critical review of the relevant literature are presented in the first part of the thesis. Clinical and analytical methods are presented in a separate section (Part II) because similar methods were used in more than one study.

The main study (Chapter 7) was designed to monitor prospectively the growth pattern, sexual maturation, nutritional status, hormonal balance and measurements of carbohydrate, lipids and protein metabolism over a period of one year in order to identify any abnormalities, their possible inter-relationships and relevance to growth. Particular emphasis was placed on the relation of energy metabolism to endocrine function which was further assessed (Chapter 8) by determining the dynamic responses of energy substrates and the metabolically important hormones to intravenous glucose, and evaluating hormone-fuel inter-relationships . Measurements of the

whole body composition and basal oxygen consumption were undertaken (Chapter 9) in order to assess the impact of chronic renal failure on body composition and to determine basal energy expenditure in relation to the metabolically active tissues. Finally (Chapter 10), the effect of diet and its manipulation on the plasma lipid profile was investigated so that deductions could be made regarding some aspects of dietary management of these children.

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PART I

GENERAL INTRODUCTION

CHAPTER I

HISTORICAL PERSPECTIVES

Uraemic Syndrome

The term uraemia was first introduced into Medical Literature in 1840 by Piorry and L'Hertier. They regarded the manifestations of renal failure as a form of poisoning of the blood due to reabsorption of urine; but it was Richard Bright (1836) who focussed clinical interest on the uraemic syndrome by his description of glomerulonephritis and the pathological changes in renal failure. Bostock (1827), who carried out the chemical examinations of serum from Bright's patients, was first to draw attention to the raised blood urea concentration in chronic renal failure. The test was crude and insensitive and Rees (1840) failed to detect urea in the blood of normal subjects.

The similarity between the uraemic syndrome and intoxications such as opium poisoning and alcoholism maintained the concept of uraemia as an intoxication. Retention of urea remained the predominant theory and various other urinary constituents were implicated during the subsequent years. In 1892 Bradford experimentally produced the uraemic syndrome clinically and chemically by excision of 75% of the kidney tissue in dogs which was later confirmed by Pearce in 1908. Renal insufficiency thus became established as the underlying mechanism of the uraemic syndrome. Further studies aetiologically broadened the category of renal failure, and uraemia came to be understood as the clinical syndrome resulting from loss of normal renal function.

The viewpoint of the uraemic syndrome as a distinct entity dates from 1918 when Volhard separated from true uraemia two syndromes which are not dependent on renal insufficiency; acute "pseudouraemia" characterised by signs of increased intracranial pressure and chronic "pseudouraemia" meant the manifestations of disturbed cerebral circulation including motor, sensory and psychic disturbances. In 1928 Oppenheimer and Fishberg described hypertensive encephalopathy overlapping Volhard's "pseudouraemia" and separated this entity from uraemia. The work of Peters (1932) contributed substantially to the understanding of electrolyte and water metabolism in renal failure. True uraemia was then separated from numerous conditions and became a more specific clinical, biochemical and pathogenetic syndrome.

The extensive studies of renal psysiology by Smith (1951) and others have shown the complexity of urine formation and it was realised that uraemia is not equivalent to the addition of urine to the blood. The concept then evolved that uraemia is the clinical syndrome caused by the biochemical alterations resulting from the loss of normal kidney tissue.

Since Paediatrics as a specialty started approximately at the same time as Bright was making his historical observation at a time when infant and child mortality was extremely high, the clinical concept of chronic renal failure in children did not arise. Golding Bird (1845), a London physician, took particular interest in children with scarletinal nephritis. He observed a rise in blood urea as a complication of the illness and considered it as contributing to the clinical problem.

At this stage, however, uraemia was recorded as an acute complication of nephritis, or as an end-stage problem preceding death in patients with established renal disease. The numerous reports of clinical observations and biochemical findings in patients with uraemia made no special mention of the syndrome as it affects children. Indeed children were considered small adults and the profound differences in body composition, metabolism and requirements of growth were not appreciated until relatively recently.

The Search for Uraemic "Toxin"

Much attention has been given to the role of urea in the past 150 years as a toxic factor causing the uraemic syndrome. The controversial role of urea was first raised by Bright in 1831 who concluded that the elevated blood urea concentration was only partly responsible for the symptoms of renal failure. The inadequacy of the urea hypothesis became apparent later and other urinary constituents and various compounds such as creatinine, indican, phenols,etc. were incriminated at one time or another. After detailed historical review Fishberg (1954) supported what Smith (1951) called the combination of ingredients theory; he wrote: "The century old search for a uraemic toxin has been fruitless. We do not yet know any single substance, the retention of which, as a result of renal insufficiency, produces uraemia - Uraemia is the complex multiplicity of mechanisms set in motion by impairment of renal function".

The worldwide experience that terminal uraemic coma is reversible by dialysis has quickened once again the search for uraemic "toxin". Guanidine compounds were implicated and Giovanetti et al. (1969) showed that chronic administration of methyl-guanidine to dogs could produce a uraemia-like syndrome. A year later Cohen (1970) reported that guanidino succinic acid was retained in renal failure in proportion to the degree of renal insufficiency and considered it as an important uraemic toxin.

Research continued into the nature of uraemic toxins, and in recent years much attention has been paid to the role of "middle molecules". It was first suggested by Babb et al. (1972) that some dialysis schedules permit the accumulation in body fluids of molecules in the range of 1000 to 2000 daltons, and that these cause some of the uraemic problems still encountered by patients receiving dialysis. Furst et al. (1975) identified groups of polypeptides of molecular weight about 1000 in the blood of a large group of uraemic patients; they bore no relationship to the glomerular filtration rate and their accumulation was considered to be due to increased production rather than simple retention.

Haemodialysis Therapy

Although the clinical use of an artificial kidney was introduced by Kolff in 1944, chronic dialysis in adult uraemic patients became possible only after the introduction of the Quinton-Scribner Arterio-Venous shunt in 1960. Subsequent development of the treatment and its combined use with transplantation altered the fate of patients with end-stage renal failure.

Haemodialysis in children is of relatively recent origin. The first successful haemodialysis in a child was reported by Mateer et al. in 1955 but its use was limited to short term dialysis for acute disorders for the subsequent ten years or so. The first pre-pubertal child started chronic dialysis in 1962 and the first children's dialysis centre was founded in Los Angeles in 1967 (Fine et al., 1968).

The primary reasons why the practice of regular haemodialysis developed so much later in children than in adults are the technical difficulties related to body size and adequate vascular access,and haemodialysis was considered at one time to be too great a psychological stress for children. Now the technical problems of dialysis in children have been solved and experience from many centres throughout the world has demonstrated that regular haemodialysis is as successful in children as it is in adults (Potter et al., 1970; Wass et al., 1977).

CHAPTER 2

GROWTH IN CHILDREN WITH CHRONIC RENAL FAILURE

Chronic renal failure is commonly associated with growth retardation (Chantler and Holliday, 1973; Schärer et al., 1976). This association was first noted with respect to rickets. Lucas (1883) was first to describe rickets in children with 'nephritis', but uraemia in childhood was considered at that time to be an acute problem and the long term effects on growth and development were not appreciated. Although fourteen years later Guthrie (1897) described short stature in children with chronic renal insufficiency it was not until Fletcher (1911) described a boy with "infantalism and polyuria" that interest in the retardation of physical development in children with renal disease was aroused. Within a few years many similar cases were described. The children became known as "renal dwarfs" (Barber, 1920) and the bone disease as "renal rickets" because of the clinical and radiological similarities to nutritional rickets (Paterson, 1920). For many years attention was focussed on rickets as the cause of short stature, since with advanced renal disease the skeletal abnormalities provided an obvious explanation (Barber, 1926; Sheldon, 1936).

The growth failure of renal disease in the absence of clinical rickets was not appreciated until relatively late (McCune, 1943; Watson and Lowrey, 1954). Although no specific explanations were offered by these authors, it was appreciated that the alteration in cellular environment and metabolism as a consequence of lost renal function lead to growth failure. It was West and Smith (1956) who were first to approach the problem systematically and considered the

possible role of various manifestations and complications of renal failure including infection, acidosis, retention of nitrogenous waste products and malnutrition. They concluded that in the absence of rickets, and chronic acidosis, calorie deficiency was the most important cause. However, as the prognosis for children with chronic uraemia was so poor that retardation of growth seemed of little importance, this crucial problem received little attention until the advent of successful treatment with intermittent haemodialysis and renal transplantation, which highlighted its impact on psychosocial rehabilitation of these children. Since then factors contributing to growth failure in children with chronic renal insufficiency have been investigated by several groups (Simmon et al., 1971; Broyer et al., 1974; Betts and Magrath, 1974) and the subject was reviewed in some detail (Bergström et al., 1964; Chantler and Holliday, 1973; Stickler et al., 1973; Lewy and New, 1975).

The factors involved in its pathogenesis are very little understood. Attention in the past was focussed on factors known to affect growth either in clinical or experimental settings, particularly bone disease and metabolic acidosis. More recently, calorie deficiency was considered to be the most important factor (Chantler and Holliday, 1973). Hormones known to regulate normal growth have been little investigated in these children. Disturbances in energy metabolism and their possible adverse effects on somatic growth have been overlooked. The search for a single cause for the growth failure

in uraemic children has, to a large extent, been fruitless. This is not surprising because normal growth is a highly complex affair and although many of its determinants are known, the mechanisms whereby these factors control growth are ill-understood. Moreover, the aetiology of growth failure of uraemia is more likely to be multifactorial and previous attempts to elucidate its pathogenesis were usually concerned with the study of a single variable in a small group of patients.

Of the various factors that are known to affect growth, nutritional, hormonal and metabolic factors play a dominant role. The pathogenesis of growth failure in children with chronic renal insufficiency will, therefore, be reviewed in relation to these three broad areas:

Nutritional Factors

(a) Basic Considerations

1. Calorie requirements, body size and growth.

In relation to body weight, the calorie requirements of children are greater than those of adults. This is because children have relatively higher basal metabolic rates, greater activity and the additional requirement of growth (Holliday, 1972). When satisfying energy needs, other nutrients which need to be excreted such as potassium, sodium and nitrogen are ingested in excess, and it is interesting to note, in this respect, that the glomerular filtration rate relative to body weight is greater in children than in adults (Holliday, 1970). In renal failure when the excretion of excess nutrients as well as the products of body catabolism can only be

achieved by altering body composition (greatly expanding extracellular volume), the impact on the child is greater. To reduce this relatively larger excretory load, anorexia is common and results in energy malnutrition which is of a greater magnitude than noted in uraemic adults.

2. Calorie intake and growth

Calorie intake in health is regulated by appetite to meet energy requirements for basal metabolism, physical activity, thermal regulation and, in children, growth. Calorie deficiency due to depression of appetite in pathological states such as uraemia will then be a limiting factor to activity and growth. Growth is very sensitive to calorie deficiency and although the cost of calories is small, estimated to be about 10% of total energy intake (Holliday, 1972), a positive calorie balance must exist for growth to take place. This is very evident in protein energy malnutrition (PEM). A mild degree of calorie deficiency affects growth in the absence of overt clinical malnutrition (McLaren, 1976). This was clearly demonstrated by the slow growth rate of Japanese children during World War II when food was rationed (Tsuchiya, cited by Holliday, 1972). It has been shown (Key et al, 1950; Ashworth et al., 1968) that the calorie cost of recovery from malnutrition to restore body composition to normal and for 'catch-up' growth to occur may be very high. It was also demonstrated (Waterlow, 1961) that the rate of weight gain in both lean body mass and fat in children recovering from protein energy malnutrition was significantly influenced by the level of calorie intake rather than that of protein.

Nitrogen balance studies (Calloway and Spector, 1954) showed that efficiency of dietary protein utilization depends on the adequacy of calorie intake. However, the efficiency of growth (weight gain per kilocalorie) seems to depend on the type of and the degree of malnutrition. Ashworth et al. (1968) have shown that the efficiency of growth was much less in the predominantly protein malnourished children than those with mainly calorie deficiency. This implies that the latter utilize energy more efficiently, perhaps due to better adaptation or that metabolic and hormonal disturbances associated with protein deficiency affect energy exchange to a greater degree.

3. Energy requirements and supply in catabolic states

Under normal conditions nitrogen excretion equals intake and for this to occur a sufficient dietary supply of calories and protein is required. A positive nitrogen balance indicates overall predominance of synthesis over degradation of body protein. The reverse is true when nitrogen balance is negative. Various studies have shown calorie needs to be greater than normal in catabolic states such as sepsis, trauma and burns (Duke et al., 1970; Hinton et al., 1971). These increased requirements cannot always be met by increased intake as illness is often associated with anorexia and disruption of the gastrointestinal tract. In these instances, endogenous energy stores such as glycogen, fat and tissue protein are mobilized for energy production. Protein sparing depends on the efficiency of metabolic adaptations to minimise protein catabolism. These adaptive processes are very

effective in normal man during starvation (Cahill et al., 1966). In contrast, during disease or trauma, the body is much less efficient in conserving its protein even with normal calorie intake (Border, 1970). It is only by providing considerably more calories than normal with adequate protein intake that nitrogen balance becomes positive and wasting is prevented (Stephens et al., 1969; Dudrick et al., 1970).

Patients with uraemia are often poorly nourished and have reduced body protein mass (Coles, 1972). The utilization of dietary nitrogen for protein synthesis is impaired and most of these patients catabolize their body protein (Hendon et al., 1958; Robson et al., 1968). Energy requirement for anabolism may be increased (Abitbol and Holliday, 1976) and the efficiency of utilization of dietary protein is improved with high energy intake (Hyne, 1972). This is of particular relevance to uraemic children as a positive nitrogen and energy balance is a pre-requisite to growth.

(b) Association of Calorie Deficiency and Growth Failure in Uraemia

1. Clinical Studies

Because of the known adverse effect of malnutrition on growth in children suffering from PEM, and since anorexia and consequent decrease of food intake is a prominent clinical feature of uraemia, it was only natural to look for a nutritional cause of the growth failure in children with chronic renal insufficiency. As stated earlier, West and Smith (1956) were first to point out the importance

of malnutrition as a major cause of growth failure in these children. Unfortunately, their study was retrospective and the subjects were a hetrogenous group of children with differing degrees of renal impairment. Their assessment of renal function was based on blood urea nitrogen levels in the majority of cases. They also used weight for height index ($\frac{\text{actual weight}}{\text{ideal weight for height age}}$) as an index of nutritional status, and a value of < 0.95 was taken as evidence of calorie deficiency. The use of such an index is fallacious as the body composition is drastically altered in chronic renal failure (Coles, 1972; Comty, 1968). The cell mass is grossly reduced and the extracellular fluid volume increased, so that simple changes in weight may not reflect nutritional status accurately. Moreover, children may have stunted growth and have reduced fat stores yet have normal weight for height (Talbot et al., 1947). Even with the use of such a crude index, a number of children in this study were growth retarded yet had an index of unity or more. Stickler and Bergen (1973) used the same index as West and Smith and found no significant difference in weight for height index between uraemic children of normal height and those with short stature. Their study was also retrospective and blood urea levels of 50 mg/100 ml or more were used as a measure of renal function. Both West and Smith (1956) and Stickler and Bergen (1973) utilized a single measurement of height for each patient and compared it to normal growth curves. This is unsatisfactory as an assessment of growth performance (Marshall, 1971). Stickler and Bergen's study also included children over the age of 11 years

without taking into account their stage of puberty when assessing their height - the error of such an omission is enormous (Marshal and Tanner, 1970).

A relationship between calorie intake and growth has, however, been demonstrated. Simmons et al. (1971) observed a reduced growth rate of five children on regular haemodialysis whose calorie intake was 67% of recommended dietary allowance (RDA) for height age compared to ten children with calorie intakes greater than RDA. They also observed an increased growth velocity in four out of five of the children with poor growth when their diet was supplemented with extra calories. However, growth increment was measured over periods which were too short to be meaningful, as was shown by Marshal (1971). Similar results were reported by Grushkin et al. (1972), Holliday (1975) and Arnold et al. (1977). Betts and Magrath (1974) studied children in chronic renal failure treated conservatively and demonstrated a correlation between energy intake and growth velocity. They also pointed out that growth retardation was only manifest when the glomerular filtration rate fell below 25 ml per $1.73m^2$, and when it was present it was associated with energy intakes of 80% of RDA or less. In seventeen children on long-term haemodialysis Broyer et al. (1974) found no close relationship between linear growth and calorie intake. More recently, Betts et al. (1977) reported their observations on a group of children with varying degrees of renal insufficiency who were given energy supplements and failed to produce evidence that reduced energy intake in children with chronic renal failure was limiting to their growth. Although there is some doubt as to

the author's interpretation of their data (Chantler et al., 1977), it is clear that whilst growth may improve with energy supplements, many children still grow poorly in spite of apparently adequate intake (Chantler et al., 1976).

2. Animal Studies

Chantler et al. (1974) demonstrated that rats rendered uraemic by sub-total nephrectomy between 40 and 70 days of age grew less and gained less weight than controls and their calorie intake was significantly decreased. They also pointed out that weight gain per kilocalorie ingested was less in the uraemic rats, and suggested a decreased efficiency in the use of energy for growth and an increase in the calories required for maintenance metabolism.

Pair feeding studies (MacDonell et al., 1973; Diaz et al., 1975) showed that the growth retardation of the uraemic rats could be mimicked by feeding the control rats the same amount of food as consumed by the uraemic rats. Moreover, calories (Adelman and Holliday, 1974) and calories plus essential amino acids (Diaz et al., 1975) resulted in accelerated growth, though in the latter study the rats were on low protein diet. Betts et al. (1976), using a different animal model, namely new born piglets to study the association of dietary intake and growth in uraemia, demonstrated similar results to those obtained with the rat model.

From the above considerations, it may be concluded that there is good evidence from animal studies implicating calorie

deficiency as a major cause of growth retardation in chronic uraemia, but clinical studies have yielded conflicting results. The growth response to high calorie diet varies; whilst growth may improve with energy supplement, many children grow poorly in spite of apparently adequate intake. One reason for this may be that results obtained at different stages of renal insufficiency and consequently different degrees of metabolic adjustment to a deranged biochemistry are not necessarily comparable. Secondly, the initial nutritional status of the child before the provision of extra energy may have bearing on the response to diet. Thirdly, and perhaps the most important factor, is that the energy requirements of man and his balance of intake and expenditure are not known (Durnin et al., 1973) and therefore the assessment of the adequacy of energy intake for growth in uraemic children is difficult when the intakes of normal children of the same size may vary considerably (Widdowson,1947). Another interesting point is whether the observed decrease in calorie intake in stunted uraemic children is the consequence of their smallness or the cause of it? Waterlow (1968) hypothesized that from the nutritional point of view smallness in size may be a useful adaptation and not necessarily an adverse effect of malnutrition since the small person needs fewer calories and less protein. In protein-energy malnutrition (PEM) when reduced food intake is due to lack of food rather than depression of appetite, Waterlow's hypothesis may be valid. By contrast the uraemic child has a poor appetite and by eating less reduces the excretory load but also reduces the energy and protein available for growth. Furthermore, nitrogen

retention for growth relieves the kidney of some of it excretory load; McCance (1959) has described growth as the 'third kidney', and in this sense growth failure in uraemic children, by reducing the protection from uraemia conferred by anabolism, could not be thought of as an adaptive phenomenon to reduce food intake but rather a result of malnutrition and other metabolic and hormonal disturbances associated with renal failure.

Understanding energy homeostasis in uraemia and the various metabolic and hormonal factors that affect anabolism will be of great value in exploring the pathogenesis of the growth failure in uraemia. No information is yet available about energy metabolism in the uraemic patient.

Chronic Acidosis

Children with renal tubular acidosis without disturbance of other renal function grow poorly (Soriano et al., 1967) and their growth improves with correction of the acidosis (Nasb et al., 1972). In a group of 41 children with chronic renal disease, West and Smith (1956) found acidosis in 76% of 21 stunted patients and none in those growing normally. Cooke et al. (1960) and several others quoted by them have also noted the relationship between growth failure and acidosis in experimental animals. However, growth retardation was not proportional to the degree of acidosis: acetazolomide administration to rats resulted in a severe metabolic acidosis despite which growth was normal. The authors suggested that the loss of fixed base consequent to the acidosis might be responsible for the growth failure observed. However, the

acidotic rats ate less than normal rats and grew less than pair-fed controls (Cooke et al., 1960; Bergstrom et al., 1964). Reduced food intake and altered nutritional efficiency, as a result of acidosis, might be an important cause of the growth failure.

Although chronic acidosis may affect growth, this is probably of limited importance in the growth retardation of chronic renal failure since children undergoing regular haemodialysis are usually not acidotic yet fail to grow. In addition,the absence of acidosis in non-dialysed children with chronic renal failure does not ensure normal growth (Bergstrom et al., 1964).

Renal Bone Disease

Osteodystrophy is a common feature of advanced renal disease, and the development and degree of bone changes seem to be related to the duration of uraemia (Beale et al., 1976). The incidence is higher in uraemic children than adults and those on maintenance haemodialysis are particularly affected (Fine et al., 1972; Potter et al., 1974).

The pathogenesis of renal bone disease is complex and not wholly understood. Factors involved in its development include disturbances in vitamin D, calcium and phosphate metabolism, and secondary hyperparathyroidism (DeLuca, 1973). A point worthy of mention is that it has been shown (Russell and Avioli, 1975) that derangements in carbohydrate metabolism in uraemia resulting in a defective energy production contribute directly or indirectly to the defect in bone metabolism.

Stunting of growth in children with nutritional rickets is well known; cure of the rickets with vitamin D improves growth. Likewise, the onset of renal osteodystrophy is associated with cessation of growth (West and Smith, 1956; Stickler, 1973). Vitamin D therapy (Dent et al., 1961) and parathyroidectomy (Broyer et al., 1974) were shown to accelerate growth in children with renal osteodystrophy. However, many uraemic children fail to grow in the absence of bone disease, and not all children with bone disease necessarily have growth retardation (Betts and White, 1976). Wass et al. (1977) found no correlation between growth velocity and degree of osteodystrophy in a group of children on dialysis. Moreover, detailed studies (Mehls et al., 1977) of skeletal changes in uraemic rats showed that longitudinal growth can proceed normally despite the presence of bone disease.

There is no doubt that severe osteodystrophy affects growth but other factors, perhaps more fundamental, are operative.

Duration of Renal Disease - Bone Maturation and Potential for Growth

Poor renal function early in life causes a more profound growth failure; stunting is more pronounced in hereditary and congenital nephropathies than in acquired lesions such as glomerulonephritis (Schärer et al., 1976). Betts and Magrath (1974) have shown that infants with renal insufficiency have a rapid fall in weight centile in the early months of life with little evidence of subsequent catch-up growth. The same group (Betts and White, 1976) further demonstrated that the greatest delay in bone maturation occurs in those children whose disease dates from

infancy rather than acquired disease but there was no extension in the delay in bone age with advancing years and deteriorating renal function. They also noted a fall of height centile with increasing bone age and that bone maturation continues despite severe osteodystrophy and growth arrest. They concluded that with increasing age, children with chronic renal failure lose potential for growth. This conclusion, however, was based on a cross-sectional study and longitudinal study will be needed to confirm this. Somewhat similar findings were reported by Broyer et al. (1974) in children on haemodialysis; the statural growth rate was found to be slower than bone maturation in early childhood, at puberty and in the presence of osteodystrophy. Thus at puberty, there is a rapid increase in bone maturation with only small increment in linear growth, with permanent loss of growth potential. This conception is supported by observations on growth of children after renal transplantation (Grushkin and Fine, 1973; Najarian et al., 1971); Saenger et al., 1974). It is accepted that the degree of skeletal maturity is a significant factor in deciding growth performance after transplantation. The data so far suggest that a bone age of 12 years or less at the time of operation is associated with satisfactory growth.

Why younger children with renal insufficiency are more growth retarded is not clear. Growth velocity is greatest early in life and therefore the earlier the 'insult' of renal disease the greater the effect on growth.

Hormonal factors

Patients with uraemia on and off dialysis display a variety of endocrine derangements. These entail abnormalities of either plasma hormone concentration or metabolism. The possible role of such disturbances in the pathogenesis of growth retardation in children with chronic renal failure will be discussed in Chapter 4.

Emotional and psychological stress

Children with chronic renal disease, particularly those on regular haemodialysis, are subjected to profound emotional and environmental stress (Korsch, 1971). The association of emotional deprivation and poor growth is well recognised and the subject is reviewed by Gardner (1972). The mechanism by which psychological stress depresses growth is controversial. Inhibition of growth hormone secretion is one possible mechanism (Powell et al., 1967) and excess cortisol production, even in small amounts, is another. However, high basal growth hormone levels have been reported in children with this condition (Krieger and Mellinger, 1971) and as will be discussed later, growth hormone concentration is raised in chronic renal failure.

Psychological stress may have its effect upon growth by depressing appetite and food intake (Whitten et al., 1969), and anorexia nervosa is an extreme example of this. Depression of appetite is a common feature in uraemic children but its cause is unknown. The central control of appetite in normal individuals is equally ill-understood and failure of growth may

be the product of hormonal and nutritional disturbances mediated through the effects of stress upon the hypothalamus. Obviously, further research in this field is required.

Other Factors

A variety of factors, known to adversely affect growth in different clinical conditions and may be present in renal failure, have been considered as possible causes of impaired growth in uraemic children. These include anaemia, hypertension, chronic infection, sodium, phosphate and potassium depletion and magnesium and zinc deficiency (West and Smith, 1956; Bergstrom et al., 1964; Broyer et al., 1974; reviewed by Chantler and Holliday, 1973). There is no evidence to suggest that any of the above mentioned factors play an important aetiological role since the absence or correction of such abnormalities is not associated with a normal growth performance in these children (Chantler et al.,1976).

CHAPTER 3

METABOLIC DISTURBANCES IN URAEMIA

"The extensive changes during renal insufficiency may disturb chemical reactions everywhere in the body and ultimately lead to a fatal dislocation of the metabolic balance".

S.E. Bradley 1946.

A wide variety of metabolic disturbances are known to occur in adult patients with chronic renal failure (Wills, 1971). These include alterations in the metabolism of carbohydrates, proteins and lipids. The pathogenesis of these abnormalities is complex and probably a sequence of altered cellular processes as a result of uraemia per se and complicated further by the effects of malnutrition so commonly superimposed on the uraemic state. Chronic haemodialysis may improve some abnormalities, but may also create new ones.

Alterations in the metabolism of the carbohydrates, proteins and lipids are of particular relevance to this thesis as they may adversely affect hormonal balance, energy metabolism and substrate availability for growth in uraemic children.

3.1. CARBOHYDRATE METABOLISM IN URAEMIA

Neubauer in 1910 first called attention to the occurrence of fasting hyperglycaemia in uraemia. This was soon confirmed by Hopkins in 1915. Shortly thereafter, Myers and Bailey (1916) demonstrated an abnormality of glucose tolerance and defined

"renal diabetes". Although these abnormalities were noted over a half a century ago, relatively little attention was paid to these problems until the advent of dialysis and transplantation. Indeed until the last decade, knowledge of the effect of chronic renal insufficiency on glucose and insulin metabolism was essentially limited to two somewhat paradoxical observations. On one hand it was believed that hyperglycaemia developed in patients with chronic renal failure (Williams and Humphreys, 1919); on the other hand, insulin requirements of patients with diabetes mellitus was shown to decrease with the development of renal insufficiency (Zubrod et al., 1951).

Although this metabolic abnormality is now a well known feature of uraemic adults there is no report to date on its occurrence in children with uraemia, and despite numerous investigations in the past fifteen years or so, there is little agreement as to its pathogenesis. This lack of agreement on the underlying defect in carbohydrate metabolism is partly the result of heterogenecity of patients studied, differing circumstances and investigative approaches.

The principal characteristics of this abnormality is excessive hyperglycaemia following oral or intravenous loading. The peak glucose concentration is elevated and there is delayed return of blood glucose to fasting levels. Such glucose intolerance occurs in greater than 50% of uraemic patients (Perkoff et al., 1958; Briggs et al., 1967; Horton et al., 1968; Hampers et al., 1968; Spitz et al., 1970; Lowrie et al., 1970). Although fasting

hyperglycaemia is present in some patients, the abnormality is more commonly evident on glucose tolerance testing.

The main pathogenetic factors implicated are:

A) Peripheral insulin antagonism

B) Abnormal pancreatic insulin secretion

C) Prolonged circulating insulin half-life

D) Abnormal hepatic glucose metabolism

E) Abnormal cellular glucose metabolism

A. Peripheral insulin antagonism:

The presence of peripheral insulin antagonism in uraemia is well established; evidence in support of this comes from the following observations:

1) The ability of exogenous insulin to lower blood glucose concentration is impaired in uraemia (Cerletty and Engbring, 1967; Westervelt and Schreiner, 1962; Hampers et al., 1966; Horton et al., 1968; Spitz et al., 1970).

2) The presence of normal or elevated fasting blood glucose in the face of a raised fasting insulin concentration (Hutchings et al., 1966; Cerletty and Engbring, 1967; Horton et al., 1968; Lowrie et al., 1970; Samaan and Freeman, 1970; Spitz et al., 1970). This observation can only be explained by insulin antagonism or by an abnormal circulating insulin. This latter possibility is unlikely since the hypoglycaemic effect of exogenous insulin is impaired.

3) Diminished and delayed fall of blood glucose following intravenous tolbutamide (Hampers et al., 1966; Cerletty and Engbring, 1967; Cohen et al., 1968; Spitz et al., 1970).

4) The most direct evidence for peripheral insulin resistance comes from the forearm perfusion studies of Westervelt (1969). These studies showed blunted glucose and phosphate uptake in muscle forearm tissue in response to exogenous insulin. They do not, however, distinguish between a circulating insulin antagonist, a defect in cell membrane transport of glucose, or a defective glucose phosphorylation.

5) Swenson et al. (1973) carried out studies in dogs before and after they were made uraemic by surgical diversions of both ureters into the vena cava thereby producing uraemia in the presence of normal kidneys. The effect of uraemia on insulin resistance was studied by the steady state plasma glucose response to continuous infusion of adrenaline, propranolol, glucose and insulin. With this approach, endogenous insulin secretion and hepatic glucose output were inhibited; similar steady state levels of circulating insulin were achieved in all dogs, and the level of the steady state plasma glucose response was a direct measure of insulin resistance. They showed that steady state plasma glucose concentration was significantly higher after uraemia had developed indicating clearly the existence of insulin resistance.

Although there seems to be little doubt that insulin resistance occurs in uraemia, the cause is far from clear. The possible causes are:

1) Abnormal form of insulin:

There is no evidence that an abnormal form of insulin is secreted by uraemic patients. The decrease in effectiveness of exogenous insulin discussed above makes this an unlikely possibility.

2) Circulating insulin antagonists:

(a) Hormones.

(i) Growth Hormone: The level of growth hormone, a well recognised diabetogenetic substance, is raised in uraemia and significant positive correlations between growth hormone and the degree of uraemia have been described (Horton et al., 1968; Wright et al., 1968; Orskov and Christensen, 1971; Reaven et al., 1974; Davidson et al., 1976). An exaggerated rise in GH levels following insulin administration has also been seen in uraemic patients. However, most investigators (Horton et al., 1968; Wright et al., 1968; Samaan and Freeman, 1970; Davidson et al., 1976), but not all (Orskov and Christensen, 1971) have found no correlation between the degree of GH elevation, either fasting or after glucose, and the degree of glucose intolerance. The role of GH in carbohydrate intolerance in uraemia remains uncertain. Its raised level may even be the result rather than the cause of this abnormality.

(ii) Parathormone : More recently, excessive secretion of parathyroid hormone has been suggested as a possible cause of glucose intolerance and insulin resistance in patients with renal failure (Lindall et al., 1971) since secondary hyperparathyroidism is a common finding in such patients, and since hyperparathyroidism seems to lead to insulin resistance and increased insulin secretion (Kim et al., 1971). It is possible that the role of parathormone could be related to its well known role in controlling serum calcium. It is also known that insulin secretion is highly dependent on calcium ions in the media bathing the islets of Lengerhan (Curry et al., 1968). However, other investigators

have not found carbohydrate intolerance in patients with hyperparathyroidism (Purnell et al., 1971). The role of increased parath-hormone secretion in the mechanism of insulin resistance in uraemia requires further study.

(iii) Glucagon: Elevated fasting concentrationsof glucagon have recently been demonstrated in chronic renal failure (Bilbrey et al., 1974; Sherwin et al., 1976). However, peripheral insulin resistance cannot be explained on the basis of hyperglucagonaemia since a diminished insulin effect on muscle characterises uraemia and glucagon has no effect on insulin-stimulated glucose uptake (Davidson et al., 1976). On the other hand suppression of glucagon secretion by glucose has been suggested as an essential component of normal glucose tolerance (Unger et al., 1970) and there is evidence that the hyperglucagonaemia of uraemia is not suppressable by glucose (Bilbrey et al., 1974). However, Sherwin et al. (1976) have demonstrated that hyperglucagonaemia did not cause glucose intolerance in normal subjects or bring about deterioration of diabetic control when insulin was available. The diabetogenic action of glucagon seemed to depend on insulin deficiency rather than absolute increase in glucagon concentration. Since haemodialysis improves glucose tolerance without altering the high plasma glucagon levels (Bilbrey et al., 1974), hyperglucagonaemia probably does not cause uraemic glucose intolerance. Further studies are necessary to clarify the role of glucagon in the abnormal glucose metabolism in uraemia.

(iv) Glucocorticoids: Adrenocorticoids antagonise the action of insulin primarily by increasing gluconeogenesis from protein (reviewed by Levine, 1964). Munk (1971) has also shown that glucocorticoids inhibit peripheral glucose uptake. Plasma adrenocorticoid levels were found to be normal or slightly elevated and to bear no correlation to the glucose intolerance in patients with chronic renal failure (Perkoff et al., 1958; Hutchings et al., 1966).

(v) Catecholamines: That the hyperglycaemic action of adrenaline is primarily the result of glycogen breakdown is well known (Katzen and Glitzer, 1968). While adrenaline raises cyclic AMP levels, insulin, although possibly indirectly, lowers the levels of this cofactor (Butcher et al., 1966). This action of adrenaline may be the basis of its insulin antagonistic properties. However, adrenaline may also inhibit insulin secretion (Kris et al., 1966). Zileli et al. (1958) found elevated plasma catecholamine levels in renal failure. They concluded, however, that this was a false increase due to interfering substances such as phenols in the method of analysis and by using a more specific method the values were found to be normal. The Oxford Group (Ledingham, 1977; Personal Communication) studying hypertension in renal failure found plasma noradrenaline concentration to be raised and adrenaline levels normal in a group of patients on regular haemodialysis for chronic renal failure. They did not, however, study glucose metabolism in their patients.

(vi) Prolactin: The diabetogenic action of prolactin is analagous to that of growth hormone (Hussay and Penhos, 1956, cited by Katazen and Glitzer, 1968). Recently elevated plasma prolactin levels have been reported in adult patients with chronic renal failure (Hagen et al., 1976), but the role this hormone plays in insulin antagonism in uraemia is not known.

(b) Metabolic end products

Renal failure is characterised by the retention of many substances, and it is possible that one or more of these is responsible for the insulin antagonism. Urea has received the most attention. Most authors have found a positive correlation between elevated urea levels and glucose intolerance (Briggs et al., 1967; Hutchings et al., 1966; Spitz et al., 1970). However, others found no such correlation (Westervelt and Schreiner, 1962; Cerletty and Engbring, 1967). Studies on the effects of urea on glucose transport and metabolism both in vivo (Perkoff et al., 1958; Hutchings et al., 1966; Hampers et al., 1966), and in vitro (Perkoff et al., 1958; Davidson et al., 1969), have yielded conflicting results and it appears that urea alone is not responsible for the glucose intolerance of uraemia. Various other substances including creatinine (Balestri et al., 1970), guadinosuccinic acid (Cohen and Horowitz, 1968), methylguanidine (Balestri et al., 1972) have been implicated, but there is no clear evidence that any of these metabolites is the cause.

The improvement of glucose tolerance by haemodialysis (Hampers et al., 1966) coupled with a longer decline in blood

glucose during an insulin tolerance test suggest that haemodialysis removes inhibitors of glucose utilisation and/or of insulin responsiveness. However, Davidson et al. (1969) found no difference in the effects of pre- and post-dialysis serum on insulin stimulated glucose uptake by rat diaphragm. On the other hand, several other studies have shown that uraemic serum does inhibit glucose uptake by rat hemidiaphragm (Balestri et al., 1972; Dzurik and Valoricova, 1970; Westervelt and Schreiner, 1962) in the absence of added insulin. This suggests that uraemia exerts an effect through non-insulin dependent cellular mechanisms. Dzurik et al. (1973) claimed the isolation of an inhibitor of glucose utilisation from serum of uraemic subjects. This peptide (mol.wt. 1000-1500) inhibits glucose utilisation in rat diaphragm, brain and kidney cortex slices as well as in human erythrocytes. This finding has not been confirmed but the molecular weight of this peptide is of interest from the point of view of uraemic toxicity, as it was suggested that 'Middle Molecules' (Mol. wt. 400 - 5000) are important uraemic toxins (Babb et al., 1972; Furst et al., 1975).

Several other factors known to cause a deterioration in glucose tolerance and insulin resistance have been suggested as possible causes of uraemic glucose intolerance. These include acidosis, malnutrition (Snyder et al., 1968), decreased total body potassium (Spergel et al, 1967), and hypocalcaemia (Lisch et al., 1973). Indeed, it is possible that there is no one cause of insulin resistance in uraemia, and that several of these factors interact to produce intolerance and insulin resistance.

B. Effect of uraemia on insulin secretion

Studies on plasma insulin response to intravenous and oral glucose have yielded conflicting results. Horton et al. (1968) and Lowrie et al. (1970) have found the early insulin response to be normal, while Hutchings et al. (1966) and Hampers et al. (1966) found it to be decreased. Insulin levels during the latter part of IVGTT have been found to be increased by all authors (Hampers et al., 1966; 1968; Horton et al., 1968; Hutchings et al., 1966; Lowrie et al., 1970; Samaan and Freeman, 1970). Similar discrepancies have been reported following oral glucose tolerance test; the early response was found to be normal (Briggs et al., 1967; Cerletty and Engbring, 1967; Hampers et al., 1968), or increased (Spitz et al., 1970; Sherwin et al., 1976). Haemodialysis increases the early insulin response to normal (Hampers et al., 1966), or even supranormal levels (Lowrie et al., 1970). On the other hand, Sherwin et al. (1976) found the response to be increased in the undialysed compared with the dialysed uraemic patients.

The removal rate of insulin from plasma is decreased in patients with renal insufficiency (see next section). Thus, the observation that the plasma insulin concentrations are equal to or greater than normal following a glucose stimulus in these patients does not distinguish between an increase in pancreatic insulin secretion rate or a decrease in insulin removal rate as being responsible. Swenson et al. (1973) attempted to separate these two effects by studying the insulin response by constant infusion of glucose in

dogs made uraemic by diversion of both ureters into the vena cava, thus producing uraemia without the loss of renal mass. Glucose concentrations were higher in uraemic dogs compared to controls, but steady state insulin responses were similar in uraemic and control dogs. Thus, the glucose intolerance demonstrated was not associated with an actual decrease in insulin response. They argued that since glucose intolerance was present, increased amounts of insulin should have been secreted to prevent the hyperglycaemia and that the insulin response may, therefore, be blunted in uraemia.

Two important considerations might explain the differences reported: First, the magnitude of the early insulin response following intravenous glucose load was overlooked by investigators failing to draw blood samples before 10 minutes, since it has been shown that insulin release in the first few minutes following I.V. glucose is the most important determinant of glucose tolerance (Porte and Bagdade, 1970), and second, failure to take into account the basal insulin levels in interpreting the insulin response to glucose. Absolute insulin levels after a variety of challenges are directly related to the basal or prestimulated level (Bagdade et al., 1967).

It is clear that glucose intolerance in uraemia is not primarily due to a defect in insulin secretion for normal responses or increases are the usual findings.

C. Effect of renal failure on insulin degradation rate

The normal kidney extracts and degrades 30-40% of the insulin carried to it (Rubenstein and Spitz, 1968; Chamberlain and Stimmler, 1967). The liver degrades at least 50% of the portal vein insulin (Blachard and Nelson, 1971). Renal failure prolongs plasma insulin half-life (O'Brian and Sharp, 1967; Spitz et al., 1970; Horton et al., 1968), and decreases its metabolic clearance rate (Fuss et al., 1974). These abnormalities could account for the protracted hyperinsulinaemia found in uraemic patients following insulin administration or endogenous insulin stimulation, as well as the decreased insulin requirements in diabetic patients who develop renal disease. On the other hand, it has been shown that even in anephric patients the insulin half-life can be shortened by haemodialysis (O'Brian and Sharp, 1967; Hampers et al., 1970). Since the liver normally degrades most of the insulin released by the pancreas into the portal circulation, a reversible defect in hepatic insulin degradation may also exist in uraemia.

While there is a reasonable agreement that insulin catabolism is impaired in uraemia, the mechanism of this metabolic change is still controversial. According to one view the slow degradation rate is due to a decrease in functioning renal tissue and to the other view it is independent of the presence of renal tissue per se and may reflect the retention of some dialysable metabolite interfering with insulin metabolism. In a study by Swenson et al. (1971) an attempt was made to distinguish between these two possibilities by calculating the fractional irreversible loss rate of labelled insulin administered to dogs when the kidneys

were intact and then immediately after bilateral nephrectomy and a few days later after the development of uraemia. Following nephrectomy the insulin irreversible loss rate fell significantly, and no further decrease was found after the development of uraemia. They concluded that loss of renal mass is responsible for the prolongation of insulin half-life in patients with renal insufficiency.

D. Role of the liver

Shoemaker et al. (1963) and Kipnis (1969) have estimated that during absorption of glucose from the intestine about two thirds is taken up by the liver. Since the liver plays such an important role in carbohydrate metabolism, it is surprising that little attention has been paid to its possible role in the abnormal glucose tolerance of uraemia. Linder et al. (1925), using the respiratory quotient as a measure of glucose oxidation, were first to suggest that impaired hepatic glycogenesis was a likely cause of carbohydrate intolerance in uraemic patients. More recent work implicating the liver was that of Cohen et al.(1961) and Cohen (1962). They described blood glucose non responsiveness to exogenous glucagon and adrenaline and suggested that there was a decrease in liver glycogen content as a result of a failure of liver glycogenesis, and that the excessive hyperglycaemia following glucose loading was a result of a reduced hepatic uptake of glucose. This has not been the general finding, however, and most authors have noted a normal blood glucose response to glucagon injection in these patients (Hampers et al., 1966; Hutchings et al., 1966; Cerletty and Engbring, 1967). Sherwin et al. (1976) demonstrated an exaggerated

blood glucose rise following glucagon administration and proposed an increased peripheral sensitivity to glucagon in uraemia which is correctable by dialysis. Furthermore normal liver glycogen content was found in uraemic rats (Boucot et al., 1960), and uraemic patients (Dzurick and Brixova, 1968). Glucose fluxes across the liver in uraemia have not yet been studied to define more precisely the hepatic glucose uptake and output in uraemia. An absolute increase in hepatic gluconeogenesis or an inability of the liver to decrease glucose production in response to insulin could also explain the glucose intolerance in uraemia. Neither of these possibilities have been explored.

E. Cellular glucose metabolism

A primary alteration in peripheral tissue glucose metabolism secondary to the metabolic changes of renal failure has been proposed as the cause of glucose intolerance and insulin antagonism (Westervelt, 1969; Davidson et al., 1969; Cohen and Horowitz, 1968). Westervelt (1969) suggested impaired phosphorylation of glucose. This view is consistent with that of Cohen and Horowitz (1968). Renner and Heinz (1972) have shown that glucose utilisation via the Krebs cycle is diminished and is increased via the pentose phosphate shunt. Uncoupling of oxidative phosphorylation occurs and the synthesis of Acetyl COA is decreased. This is consistent with work of Galloway and Morgan (1964) who found elevated pyruvate levels in their uraemic patients and suggested a metabolic block in pyruvate utilisation. However, Ganda et al. (1976) reported normal plasma pyruvate

concentration in their patients. Metcoff et al. (1978), using the circulating neutrophil as a cell model, demonstrated that the decrease in blood glucose levels post-dialysis was associated with significant increases in the activities of the regulatory glycolytic enzymes: Glucose-6-phosphate dehydrogenase (G-6-PD), phosphofructokinase (PFK) and Pyruvatekinase (PK).

The effect of uraemia on the activities of the enzymes of the glycolytic, hexosemonophosphate, and glycogen cycle pathways has not received much attention and data reported (Dzurick et al., 1969; Boucot et al., 1960; Mannan et al., 1975; Maier et al., 1973) has been conflicting and difficult to interpret as the enzyme activities and/or intermediate metabolites were studied in different tissues under different conditions.

Effect of haemodialysis on glucose intolerance of uraemia

It is generally accepted that haemodialysis improves glucose tolerance in patients with uraemia (Hampers et al., 1966; 1970; Lowrie et al., 1970; Spitz et al., 1970). However, Reaven and his colleagues have pointed out that this apparent improvement was due to non-specific methods used for glucose determination (Swenson et al., 1973). They found no improvement on oral glucose tolerance tests using a specific glucose-oxidase method. They pointed out that the use of non-specific methods that do not discriminate between glucose and other reducing substances overestimates the apparent improvement in glucose tolerance that follows dialysis. Davidson et al. (1976) reported similar observations. They also

considered the glucose intolerance in uraemia to be modest. On the other hand, there are studies which have used specific methods for measuring blood glucose which demonstrated glucose intolerance in uraemic patients (Westervelt and Schreiner, 1962; Cerletty et al., 1967; Horton et al., 1968; Orskov and Christensen, 1971).

The insulin response to a glucose load rises significantly with chronic dialysis (Alfrey et al., 1967; Hampers et al., 1966; 1970; Spitz et al., 1970), and it may even be supranormal (Lowrie et al., 1970; Lindall et al., 1971). This rise is particularly striking in the first ten minutes of an intravenous glucose tolerance test and correlates, in some studies, with improvement in glucose disappearance rate (Hampers et al., 1966; Lowrie et al., 1970). Haemodialysis also decreases the high fasting plasma insulin concentration and shortens the insulin degradation rate (Hampers et al., 1970; Ganda et al., 1976).

The mechanism by which haemodialysis alters glucose and insulin metabolism is not known. The increased insulin response to glucose stimuli after adequate dialysis suggests the removal of a dialysable inhibitor of insulin release. It has also been suggested that dialysis removes inhibitors of glucose uptake (Dzurik et al., 1973). Davidson et al. (1969), however, found no difference in the effects of pre and post dialysis serum on insulin stimulated glucose uptake by rate hemidiaphragm. Dialysis may improve glucose metabolism by the removal of inhibitors of key enzymes in the glycolytic pathways (Metcoff et al., 1978).

Summary and Conclusions

Carbohydrate intolerance with hyperinsulinism is a well established feature of chronic renal failure. This impairment is the result of renal failure or some effect of it, and is not simply the consequence of malnutrition or simple electrolyte imbalance. Peripheral insulin resistance, the mechanism of which is not known, and decreased degradation rate of circulating insulin - primarily due to loss of renal tissue - would appear to be the principle pathogenetic factors. There is little evidence which implicates a primary defect in pancreatic insulin release as a cause of the glucose intolerance, but there is the possibility that uraemia inhibits the normal compensatory response of β-cells to hyperglycaemia. The complexity of the dynamics of normal insulin release from β-cell coupled with the profound disturbances in cellular metabolism in uraemia, makes the state of insulin secretion in uraemia difficult to assess. Haemodialysis, according to most authorities, improves glucose tolerance in uraemic patients but does not normalise it.

Although the glucose intolerance of uraemia and its associated hyperinsulinism does not have obvious clinical manifestations, the defective energy production which may result from such abnormalities in glucose metabolism may have a direct or indirect effect on cellular energy metabolism. It is not known what influences these alterations have on other aspects of metabolism such as protein, lipids and other hormones.

Understanding of these interrelationships is of obvious importance and they may be implicated in the growth failure and tendency to catabolism characteristic of renal failure; this dissertation is primarily concerned with this aspect of the problem.

3.2. LIPID METABOLISM IN URAEMIA

Lactescence of the serum from patients with renal disease was noted over one hundred and sixty years ago (Blackall, 1813; Bostock, 1827). However, these early observations were on 'dropsical' patients likely to have the nephrotic syndrome which is classically associated with hypercholesterolaemia. Chauffard et al. (1911) and Ashe and Bruger (1933) described elevated serum cholesterol levels in patients with chronic renal failure which returned to normal or below normal levels as the uraemia progressed. This fall was considered to be due to cachexia (Ashe and Bruger, 1933).

Although increased blood lipid levels have been observed in experimental renal failure for many years (Johnson et al., 1951), only recently the lipid abnormalities in adult patients with chronic renal failure, both treated conservatively or by regular haemodialysis, have been described (Bagdade et al., 1968; Arora et al., 1973; Gutman et al., 1973; Ibels et al., 1975; Daubresse et al., 1976). Hypertriglyceridaemia is the prominent feature and plasma cholesterol concentrations are usually found to be normal though occasionally increased (Kaye et al., 1973; Persson, 1973; Ibels et al., 1975). There are considerable interstudy variations in the reports published due primarily to differences in the degree of renal impairment, mode of therapy, dialysis schedules and dietary information in the patients studied. A wide variation in control values were also used in assessing the degree of plasma lipid abnormalities in these patients. It is, therefore, difficult to compare these studies or discriminate between them.

Recently, two studies on plasma lipid levels in children on regular haemodialysis for chronic renal failure have been published (Pennisi et al., 1976; Broyer et al., 1976). The findings in both studies were similar to those reported in adults with hypertriglyceridaemia as the main feature and raised plasma cholesterol in some but not all of the patients.

Lipoproteins and triglycerides

Elevated plasma triglycerides are present in the majority of uraemic patients, whether they are managed conservatively or on regular haemodialysis and appear to persist in most patients after a successful renal transplantation (Ibels et al., 1975; Bagdade et al., 1976). Roodvoets et al. (1967) first demonstrated that the raised triglyceride level in uraemia was associated with an increase in the plasma pre-beta lipoproteins and this was confirmed by others (Bagdade et al., 1968; Ibels et al., 1975; Kaye et al., 1973; Cattran et al., 1976). It is now well established that the hypertriglyceridaemia of uraemia is due to an increase in the plasma concentration of triglyceride - rich, very low density lipoprotein (VLDL). This corresponds to Type IV according to Fredrickson's classification (Fredrickson et al., 1967). Fredrickson and his colleagues considered that this type of lipoprotein pattern, which may be either primary or secondary, is characteristic of endogenous hyperlipidaemia, and is typically carbohydrate induceable and accompanied by glucose intolerance. This phenotype may occur as a result of increased hepatic triglyceride synthesis, diminished removal of triglyceride from plasma or a combination of both. However,

recent analysis of plasma lipoprotein fractions in undialysed and dialysed uraemic patients (Bagdade et al., 1976, 1977; Norbeck et al., 1976) revealed more extensive abnormalities in the composition of the three major lipoprotein classes (VLDL, LDL, HDL): The triglyceride and cholesterol concentrations in VLDL were increased, whilst their normal ratio for this lipoprotein class was maintained. In LDL, the concentration of triglycerides was increased, whilst that of cholesterol was low. The LDL was, therefore, more triglyceride-rich than normal indicating a disturbed triglyceride metabolism. The cholesterol content of HDL was extremely low but HDL triglyceride was only slightly raised.

Mechanism of hypertriglyceridaemia of uraemia

The exact mechanism responsible for the elevated plasma triglyceride concentration is not known. However, two possible mechanisms were proposed:

1. Increased hepatic triglyceride synthesis
2. Decreased plasma triglyceride clearance.

Bagdade et al. (1968) and Bagdade (1970) suggested that the increased plasma triglyceride concentration was the result of both increased TG production by the liver and impaired removal from plasma - a combination they termed 'mixed lipaemia'. They considered the increased hepatic TG synthesis to be the consequence of the hyperinsulinaemia found in these patients. This was based on the indirect evidence of a positive correlation between plasma TG concentration and plasma insulin level. Since hyperinsulinaemia is known to be associated with increased hepatic TG synthesis in non-

uraemic subjects (Reaven et al., 1967; Bierman and Port, 1968; Eaton and Nye, 1973; Olefskey et al., 1974), and since a decrease in the levels of both plasma insulin and TG concentration was noted following intensive dialysis, a causal relationship was suggested. Although this remains a viable possibility, there is as yet no direct evidence that elevated insulin levels alone increase hepatic triglyceride production.

Studies on the kinetics of triglyceride metabolism in uraemic patients produced conflicting results. Cramp et al. (1976) studied TG synthesis rate in uraemic adults and reported raised plasma TG concentration, a diminution in the fractional turnover rate and an increase in the total turnover rate. They suggested hepatic over-production of triglycerides. However, Cattran et al. (1976) found the TG turnover rates to be less than the values predicted for comparable plasma TG concentration in non-uraemic subjects and implied a defect in the plasma TG removal system.

The first step in the removal of triglyceride from plasma and its deposition in adipose tissue is hydrolysis. This is catalysed by the enzyme lipoprotein lipase (LPL). Post heparin lipolytic activity 'PHLA', which is an indirect measure of tissue lipoprotein lipase and hence TG removal capacity, is reduced in uraemia (Bagdade et al., 1968; Gutman et al., 1973; Daubresse et al., 1976). The decrease in PHLA has been interpreted by various authors as an indication of a defect of plasma TG clearance system. This plasma lipolytic activity reflects more accurately the release of LPL from a number of different tissue sources into plasma after heparin administration (Krauss et al., 1974) and hence may not reflect

quantitatively adipose tissue LPL which is believed to be the principal mediator of TG removal (Robinson, 1965). Moreover, PHLA does not differentiate between absolute deficiency of tissue LPL, resistance to heparin action, or the presence of an inhibitor of LPL in uraemia. However, Persson (1973) reported a low level of LPL in fat cells of uraemic patients and considered this as an important factor causing hypertriglyceridaemia in uraemic patients. More recently, Murase et al. (1975) demonstrated an inhibitory effect of plasma from uraemic patients on LPL activity in vitro and suggested the presence of circulating inhibitor of LPL which is not dialysable. In support of the hypothesis of a reduced plasma TG clearance, Ibels et al. (1976) demonstrated a decreased clearance of infused TG emulsion 'Intralipid' in uraemic patients.

It has also been suggested (Bagdade, 1975) that the peripheral insulin resistance known to occur in uraemia may be a factor in the lowering of LPL activity, thus reducing TG removal, since insulin is probably required for normal LPL activity (Elkeles, 1973). LPL activity is also reduced in malnutrition, thyroid deficiency and corticosteroid excess. These three factors, particularly malnutrition, occur in uraemia.

Influence of haemodialysis on the hypertriglyceridaemia

Haemodialysis may affect plasma TG levels in a number of ways: glucose and acetate can cross the dialysing membrane and contribute to increased hepatic TG synthesis. However, glucose-enriched and glucose free dialysate seems to have no appreciable effect on plasma

TG concentration (Hubner et al., 1971; Daubresse et al., 1976). Systemic heparanisation is another important factor because heparin activates tissue LPL in normals, but no difference in plasma TG was found after systemic or regional heparinisation in uraemic patients (Daubresse et al., 1976). Moreover, if the presence of hypertriglyceridaemic factor is postulated, haemodialysis does not seem to remove it as high plasma TG levels are as evident in the dialysed as they are in the undialysed patients or even higher. However, Bagdade (1970) reported a decrease in plasma creatinine, insulin and triglycerides in one patient after intensive haemodialysis. Similar observations were reported by Gutman et al. (1973). It may be that the amelioration of the uraemic state by more intensive dialysis improves lipid metabolism as it does improve other metabolic disorders, particularly carbohydrate metabolism (Hampers et al., 1973).

Plasma cholesterol

Disturbance in cholesterol metabolism is classically seen in association with the nephrotic syndrome. In non-nephrotic patients with chronic renal failure plasma cholesterol concentration is usually found to be within normal range. However, increased plasma cholesterol levels have been reported (Cohen and Lindall, 1969; Kaye et al., 1973; Persson, 1973). Broyer et al. (1976) found 38% of children on haemodialysis to have high plasma cholesterol levels. As stated above, the predominant electrophoretic lipoprotein pattern is type IV but occasionally types IIa and IIb with

increases in both plasma TG and cholesterol concentrations are found. The concentration of the normally cholesterol-rich LDL was reported to be normal but with increased TG content (Bagdade et al., 1976; Norbeck et al., 1976). Ibels et al. (1975) found an increase of LDL in their patients, but with normal cholesterol concentration. The VLDL cholesterol content is increased (Bagdade et al., 1976; Norbeck et al., 1976), but HDL cholesterol content is very low (Norbeck et al., 1976; Bagdade et al., 1977).

Role of diet

The concentration of plasma triglycerides in normal individuals is readily increased by diets which are rich in carbohydrates. The magnitude of this increase and its duration are governed by factors such as the amount and the type of carbohydrates, age, sex, presence of obesity and genetic predisposition (Fredrickson et al., 1967). The causal mechanisms have not been established although published reports suggest that increased formation of triglycerides in the liver is of greater importance than diminished removal from the plasma (Reaven et al., 1965; Nestel, 1966). Insulin is thought to play a major role since the insulin response to the ingestion of carbohydrates appears to be significantly correlated with the degree of hypertriglyceridaemia (Farquhar et al., 1966; Reaven et al., 1967). Since circulating plasma free fatty acids are a major source of plasma TG, excessive synthesis of FFA from carbohydrates has also been considered as a possible causal factor. There is no support for this, however, apart from occasional

findings of raised plasma FFA in some hypertriglyceridaemic states. The consumption of saturated and polyunsaturated fat is also known to influence the plasma triglyceride concentration (Ahrens et al., 1957).

Although it is a potential cause of the endogenous hypertriglyceridaemia of chronic renal failure, carbohydrate intake has not been found to correlate with plasma triglyceride levels in uraemic patients (Bagdade et al., 1968; Ibels et al., 1976; Cattran et al., 1976). This lack of correlation does not exclude entirely the possibility that the increased carbohydrate intake often prescribed for patients with renal failure may be important. Both Pennisi et al. (1976) and Broyer et al. (1976) demonstrated a positive correlation between the percentage calories derived from carbohydrates and plasma TG levels. They also reported a positive relationship between carbohydrate intake either related to body weight (Pennisi et al., 1976) or to recommended daily allowance (RDA) (Broyer et al., 1976) and plasma TG concentrations but this relationship must be viewed with caution. Expression of nutrient intake in relation to body weight when the population studied by them included young children and young adults is complicated by the fact that in relation to body weight, young children have a considerably higher intake (Widdowson, 1947) and if age is not taken into account, erroneous conclusions may be reached. It is, therefore, more appropriate to refer food intakes to surface area or to RDA. However, Broyer et al. (1976) in calculating the RDA for carbohydrates assumed that carbohydrates provide 60% of calories in the normal diet, a

figure much higher than is accepted in the UK (40-45%). More recently, Sanfelippo et al. (1977) reported significant reductions in plasma TG levels after feeding their uraemic patients diets low in carbohydrate and high in polyunsaturated fat. Unfortunately, it is not known from their study which of the two variables in the diet altered the TG levels. The lowering of plasma TG was associated with a decrease in TG production rate and plasma insulin response. They suggested a defect in plasma TG clearance as the prime cause of the raised TG levels because the patients had higher plasma TG levels than did normal subjects at any given TG production rate. However, amelioration of hypertriglyceridaemia could have been obtained by a decrease in TG production rate with low carbohydrate diets.

Clinical significance of plasma lipid abnormalities in uraemia

Epidemiological studies have clearly defined the relationship of cardiovascular disease and hyperlipidaemia (Kannel et al., 1971; Carlson and Böttinger, 1972); raised levels of VLDL (Carlson et al., 1975) and low levels of HDL (Miller and Miller, 1975; Carlson and Ericsson, 1975) are associated with ischaemic heart disease.

An increased incidence of atherosclerosis and premature cardiovascular disease in adult patients on regular haemodialysis has been reported recently (Lindner et al., 1974; Lowrie et al., 1973; Brunner et al., 1972). The occurrence in chronic renal failure of the three lipoprotein abnormalities,

1) increased VLDL, 2) increased LDL, and 3) decreased HDL, may be a major contributing factor in the rapid development of atherosclerotic vascular disease in these patients. Many children with chronic renal failure are now being treated by regular haemodialysis in preparation for renal transplantation and are often encouraged to take carbohydrate and fat supplements in their diet in an attempt to improve statural growth. An evaluation of plasma lipid profile, particularly in relation to diet, is obviously important for rational management of these children.

FREE FATTY ACIDS

General considerations

Free fatty acids (FFA) are an important metabolic fuel supplied by the blood to peripheral tissues. They can be used for energy production by many different cells in the body, (and probably are the major source of fuel for cardiac and striated muscle (Fritz, 1961)) or can be stored in adipose tissue as triglyceride until needed.

It had been realized long ago that most stored energy is in the form of fat. This maximises efficiency, adipose tissue having very little intracellular water and thus having the most calories available per gram of tissue. Triglycerides are hydrolysed to glycerol and FFA. Glycerol is primarily converted to glucose in the liver (gluconeogenesis) and accounts for only a small fraction of the energy requirement; FFA become the major energy source being oxidised to CO_2 and water. Important metabolites of FFA oxidation in the liver are ketone bodies, which become a substitute energy substrate during starvation (Cahill et al., 1966).

The pool size of plasma FFA is very small, but because of the very rapid turnover large quantities of FFA can be transported in the course of a day from one part of the body to another bound to plasma albumin (Issekutz et al., 1967). The caloric value of the FFA flux is, therefore, very large amounting to two thirds or more of the total caloric expenditure of an average man (Dole and Rizzack, 1961).

Hormones play an important role in the regulation of FFA release from adipose tissue (Siddle and Hales, 1975). Growth hormone, glucagon, TSH, thyroxine and catecholamines stimulate the triglyceride lipase activity in adipose tissue and increase FFA mobilisation; insulin and some prostaglandins inhibit the lipase activity and decrease FFA mobilisation. The sympathetic nervous system also plays an important role but quantitative information is lacking at the present time.

Insulin is a potent antilipolytic hormone (Butcher et al., 1966; Siddle and Hales, 1975), and it is generally accepted that it exerts its antilipolytic effect by lowering intracellular cyclic AMP levels. Insulin also antagonises the lipolytic effects of other hormones such as glucagon, catecholamines and ACTH (Butcher et al., 1966). The interrelationships between cyclic AMP, hormones and lipolysis are very complex, but insulin appears to play a dominant role in the control of fat mobilisation. Being an anabolic hormone, it enhances lipogenesis by promoting glucose uptake by adipose tissue and stimulating re-esterification of FFA to triglycerides. A decrease in insulin levels has a permissive effect on fat mobilisation and liberation of FFA in excess as energy substrate in time of need, e.g. starvation (Cahill et al., 1966).

The concentration of FFA in the blood is related to FFA release into the circulation which in turn is determined by the balance between triglyceride breakdown during lipolysis and the rate of re-esterification of fatty acids. The latter process is dependent on glycerol phosphate derived from glucose. The uptake and oxidation

of FFA by tissues is a function of their concentration in the plasma (Issekutz et al., 1967). The amount of glycerol released is a measure of that formed by hydrolysis of triglycerides, and since glycerol utilization by adipose tissue is negligible, lypolysis is usually measured by the amount released into the circulation (Steinberg and Vaughan, 1965).

Free fatty acid in uraemia

Little information is available regarding fatty acid metabolism in uraemia. Fasting plasma concentrations have been reported by various investigators to be within the normal range (Hampers et al., 1966; Spitz et al., 1970; Daubresse, 1976). Others (Persson, 1973; Ghosh, 1973) found reduced plasma FFA levels in their patients. Raised levels were reported in patients with acute uraemia and during haemodialysis (Losowsky and Kenward, 1968; Mydlik et al., 1976). The increased FFA levels during dialysis are likely to be due to heparin which stimulates plasma lipolytic activity and, therefore, do not reflect the effects of uraemia on FFA metabolism. Because of their extreme lability and rapid turnover rate, single untimed measurements of plasma FFA concentration do not represent accurately the rate of FFA release from adipose tissue. However, oral and intravenous glucose produced a fall in plasma FFA concentration which was of greater magnitude and duration in uraemic patients than in a group of normal subjects (Roth et al., 1973; Losowsky and Kenward, 1968). This response suggests that the effects of hyperglycaemia and insulin on adipose tissue are

preserved and may even be enhanced in uraemia. Unfortunately, in spite of the close relationship which has been shown to exist between glucose and FFA metabolism (Randle et al., 1963) and the major contribution of FFA to the body's total energy expenditure, studies of the kinetics of this important metabolic fuel have not been undertaken in uraemia.

3.3. NITROGEN METABOLISM IN URAEMIA.

Patients with chronic renal failure develop a number of manifestations of abnormal protein metabolism. These include reduction in body protein mass, particularly muscle protein, negative nitrogen balance and disturbances in amino acid metabolism. Because uraemic patients are often subnourished as a result of prolonged anorexia or deliberate dietary protein restriction, it proved difficult to ascertain whether the abnormalities in nitrogen metabolism are attributable to uraemia per se, to malnutrition, or to an interaction between uraemia and malnutrition. In fact, the general malnutrition in uraemia depends on several factors which are not easily separated and appear partly as a result of poor dietary intake, gastrointestinal disturbances, and increased demands of energy and perhaps also of nitrogen, besides various derangements of intermediate metabolism.

NITROGEN BALANCE IN URAEMIA

Many investigators have reported a persistently negative nitrogen balance in patients with advanced chronic renal failure (Hyne et al., 1972; Kopple and Swenseid, 1974), but it is still uncertain whether insufficient intake of energy and nitrogen, poor absorption, or interference with protein synthesis or degradation is the prime cause.

Major factors which influence nitrogen retention

(a) Nitrogen intake

There is no concensus of opinion on the minimal protein intake required by uraemic patients and the optimal protein and energy requirements have not yet been determined with any degree of accuracy. Even in normal individuals the ideal protein requirements are debatable. Estimates of minimal nitrogen requirement for maintenance of nitrogen balance in uraemic adults have varied from 3.1 - 5.6 grams per day (Shaw et al., 1965; Ford et al., 1969). It is doubtful whether these figures differ significantly from 3.1 - 4.2 grams minimal nitrogen requirement for normal individuals (Bricker et al., 1949; Rose and Wixon, 1955). Giordano (1963) and Giovanetti (1964) demonstrated that uraemic patients could re-utilise urea for protein synthesis if essential amino acids constituted the bulk of their dietary protein, suggesting that the nitrogen requirements of uraemic patients may be reduced. Using high quality protein diets Giordano et al. (1967) and Berlyne and Hocken (1968) observed that uraemics may be able to maintain a nitrogen balance on lower protein intakes than normal subjects can. However, results obtained by Kopple et al. (1968) and Ford et al. (1969) failed to support this conclusion although the enlarged nitrogen pool in uraemia, mainly coming from urea and non-essential amino acids can provide supplementary nitrogen for protein synthesis under reduced protein intake. Such recycling of nitrogen may be insufficient for anabolic purposes (Walser, 1974; Richards, 1975) and data obtained from experimental animals (Young et al., 1973) suggest that protein requirements may be higher in uraemia than normal despite probable reutilisation of non amino

nitrogen, so that a low protein intake will cause more marked reduction in protein synthesis and increase body protein depletion. The provision of ketoacid analagous of most essential amino acids have been shown to produce more efficient nitrogen utilisation and maintain nitrogen balance even if the protein intake is low (Walser et al., 1973). But the improvement in protein anabolism so realised seems still insufficient for body economy; a considerable amount of analogue compounds are not utilised for synthetic purposes but undergo degradation in the body, so that benefits for limited periods only have been achieved. In order to attain nitrogen equilibrium, the diet must supply, in addition to essential amino acids, sufficient nitrogen for the in vivo synthesis of non-essential amino acids. Such a requirement will further reduce nitrogen available for anabolism if dietary protein is restricted.

(b) Energy requirements for maximal protein utilisation

A crucial factor influencing the efficiency of utilisation of the intake nitrogen is the non protein energy content of the diet. It is generally recognised that tissue protein synthesis can proceed effectively only if there is an adequate intake of energy (Munro, 1964). The protein energy relationship was emphasised by Calloway and Spector (1954) who showed that in normal individuals a limiting energy level could be defined beyond which increasing the energy intake is of no advantage. They found that an intake of 3 grams nitrogen daily resulted in maximum nitrogen retention with approximately 900 calories, and that no further nitrogen retention occurred on increasing the energy. In uraemic patients, on the

other hand, nitrogen retention continues to improve beyond what is expected with additional increments to the energy intake (Hyne et al., 1972). This suggests that the uraemic patient is somehow more dependent on the level of calorie intake than a normal subject and that there is an additional demand for energy over and above that required in normal individuals. The reason for this is not clear but it may be the necessity for greater utilisation of endogenous non protein nitrogen which depends on the availability of ATP.

(c) The effect of metabolic rate

Alteration in energy expenditure adds another dimension to the interrelationships between nitrogen intake and energy intake and nitrogen balance. It is well known that an increase in metabolic rate in hypermetabolic states such as infection, trauma or burns is associated with negative nitrogen balance. However, the provision of energy sufficient to cover energy expended, along with a normal nitrogen intake, fails to establish positive nitrogen balance as shown by Coleman and Dubois (1915) more than 60 years ago in patients with typhoid fever. Soroff et al. (1961) demonstrated the effect of metabolic rate on protein efficiency and nitrogen balance in a study of a group of hypermetabolic burn patients receiving energy sufficient to satisfy metabolic demands (100 - 120% of BMR measured by indirect calorimetry) and a constant intake of normally adequate dietary nitrogen. They found nitrogen balance became more and more negative as the metabolic rate increased and, in order to achieve nitrogen equilibrium, nitrogen intake had to be increased

to approximately double the quantity normally required at any level of energy intake. This observation suggests that the normal relationship between energy and nitrogen intake as described by Calloway and Spector (1954) is altered in hypermetabolic states. It is evident that not only are the energy demands increased in these patients but also nitrogen requirements, both in absolute terms and in relation to energy intake, the increase in metabolic rate is the underlying cause.

Basal energy expenditure of malnourished animals (McCance and Mount, 1960) and children (Ablett and McCance, 1969) is low and rises to normal after recovery. Although the cause of the low BMR has not yet been elucidated, it seems to be an adaptation to low energy and protein intake. Ashworth (1968) has shown that young Jamaican adults on low-calorie intakes for long periods of time adapt and maintain energy balance and body composition by developing a lower maintenance energy requirement and a lower rate of energy expenditure for physical activity.

The relationship between metabolic rate and growth (as assessed by body weight) is interesting. Montgomery (1962) noted that the weight of malnourished children did not increase unless the calorie intake consistently exceeded the basal metabolic rate, and that the greater the weight deficit the higher was the BMR during recovery and the higher the calorie requirements for weight gain.

Inadequate food intake is common in uraemic children and may be a factor in their poor growth. This may be that uraemia increases

basal calorie requirements either by decreasing the efficiency of food utilisation, or by increasing basal energy expenditure making the requirements for energy and nitrogen balance much higher than normal and thereby limiting growth. No observations on the basal energy expenditure of children with chronic renal failure have yet been reported and the influence of the BMR, if any, is not known.

IMPACT OF URAEMIA ON NUTRITIONAL STATUS

Poor dietary intake is common in patients with uraemia and there are striking similarities in many indices of nutrition between chronic renal failure and protein-energy malnutrition syndrome (PEM) (Chantler and Holliday, 1973). However, in uraemia, factors other than deficient food intake may act directly to influence the nutritional status. Thus nutritional state assessment alone will not provide information regarding the cause of the disorder and this is obviously needed before appropriate action can be taken to restore the balance.

Assessment of nutritional state

Nitrogen balance measurements, discussed above, do not provide a reliable index of the nutritional state since (a) nitrogen requirements may vary with the degree of protein depletion (Allison, 1951), (b) overall balance may not reflect balance in critical organs or tissues (Shear, 1970). Furthermore, they are difficult to perform with any accuracy particularly in patients on dialysis because of nitrogen losses in the large volumes of dialysate.

Body composition

Gross body composition reflects previous nutrition. In nutritional deficiency the composition will change but will revert to normal with treatment. Measurements of whole body composition in adult uraemic patients both on and off dialysis (Comty, 1968; Coles, 1972) showed changes similar to those reported in subjects with protein-energy malnutrition (Waterlow and Alleyne, 1971). In both conditions there is a reduction in body fat, fat-free solids and lean body mass; body water is increased relative to body weight due to an excess of intracellular fluid. Wilson et al(1971) studied one uraemic child on dialysis and reported increased extracellular fluid and decreased cell mass. These changes in body composition may be entirely due to malnutrition (Coles, 1972) but recent experimental evidence (Adelman, 1977) suggests that uraemia, independent of nutrient intake, may affect body composition. This author found total body water and extracellular fluids to be increased and total body solids decreased in uraemic rats compared with pair-fed controls.

Precise indices of protein deficiency are lacking. Serum albumin concentration, a crude index of protein malnutrition is depressed in some uraemic patients even in the absence of heavy proteinuria and was considered to represent dietary protein deficiency (Coles, 1972). Serum transferrin concentration is a more sensitive index of protein-energy malnutrition (McFarlane et al., 1969). Subnormal levels of transferrin have been found in uraemic patients with apparently adequate food intake and normal plasma albumin concentration, and were equally low in dialysed and non-dialysed patients (Young and Parson, 1970; Ooi et al., 1972).

Thus, despite improvement in dietary intake that usually follows regular dialysis, subclinical protein-energy malnutrition may persist.

Plasma concentrationsof complement proteins are sensitive indicators of nutritional state. Several components of the system including C_3 were found to be decreased in uraemic patients and reverted to normal with essential amino acid supplementation (Heidland and Kult, 1975). Young et al. (1975) have shown that the plasma levels of C_3 and that of transferrin were dependent on the availability of essential amino acids in the diet and emphasised their value in the assessment of dietary intake in chronic renal failure.

A more subtle but striking similarity between chronic renal failure and protein-energy malnutrition syndrome is the plasma amino-gram. The abnormalities in the plasma amino acid profile in uraemia (vide infra) is at least partly due to protein deficiency, as similar changes are also seen in patients with Kwashiorkor as well as in normal subjects maintained on low protein diet (Whitehead and Dean, 1964; Swendseid et al., 1968).

PLASMA AMINO ACIDS IN URAEMIA

Many plasma amino acid levels are altered in chronic renal failure even after treatment by haemodialysis (Gulyassy et al., 1970; Young and Parson, 1970; Kopple and Swendseid, 1975). However, there are many discrepancies in these reports which may relate to differences in dietary intakes, degree of renal impairment, dialysis procedures,

or methods of plasma amino acid analysis. Abnormalities for which there is some consensus are decreased levels of essential amino acids particularly the branched-chain amino acids valine, leucine and isoleucine; tyrosine and lysine are usually decreased, and cystine is increased. Plasma levels of non- essential amino acids are usually normal or increased, particularly glycine and citrulline. One and 3-methylhistidine, normally present in trace amounts in plasma, are increased. The ratios in plasma of essential/non-essential amino acids, valine/glycine, tyrosine/phenylalanine, are characteristically reduced. Alterations in the concentration of phenylalanine and histidine (considered as an essential amino acid in uraemia) vary from study to study.

Pathogenesis of plasma amino acid abnormalities

The mechanisms underlying the plasma amino acid abnormalities are not well understood. Published reports indicate a multifactorial cause and the following possibilities are considered important.

(1) Nutritional factors

Alterations in plasma amino acid concentrations in children suffering from protein-energy malnutrition or individuals on low protein diets are well documented (Arroyave et al., 1962; Swendseid et al., 1968). In these conditions the essential amino acids particularly the branched-chain amino acids,valine, leucine and isoleucine are reduced and the non-essential amino acids are normal or increased. The plasma valine concentration is particularly sensitive to low protein intake, while glycine is usually increased

and, therefore, a reduced valine/glycine ratio is characteristic of protein malnutrition. As anorexia is a common feature of chronic renal failure and often leads to reduced spontaneous food intake (Holliday, 1972), the observed similarities between plasma amino acid profiles in uraemia and/PEM lead to the assumption that malnutrition underlies the plasma amino acid abnormalities in uraemia. However, while the disordered aminogram in protein energy malnutrition reverts to normal on feeding,the abnormalities in uraemic patients seem to persist after apparently adequate protein and energy intake attending regular haemodialysis. In an elegant study, Kopple and Swendseid (1975) attempted to separate the effects of uraemia per se from that of protein malnutrition on plasma amino acid pattern in uraemic patients and concluded that both uraemia and protein intake seem to affect plasma amino acid levels; some concentrations of amino acids are abnormal independent of protein intake, other amino acids differ from normal only as a result of low protein intake. They also demonstrated that the response of many amino acids to protein restriction is abnormal in uraemic patients compared to normal subjects. Uraemia per se, therefore, directly affects protein and amino acid metabolism. Specific enzyme defects, such as the impairment of the activity of the enzyme phenylalanine hydroxylase with reduced tyrosine formation and decreased tyrosine/phenylalanine ratio has been demonstrated in uraemia (Young and Parson, 1970). Other less specific metabolic defects may involve reduced efficiency of protein and energy utilisation for anabolic purposes as the requirements of both seem to be increased in uraemia.

The amino acid abnormalities may be related to the disordered carbohydrate and fat metabolism, already discussed, and/or changes in the hormonal milieu in uraemia. Such interrelationships have not been explored.

(2) Dialysis losses

Haemodialysis does not directly produce substantial losses of albumin or globulin, but large losses of free and conjugated amino acids occur (Rubini and Gordon, 1968; Kopple et al., 1973). However, the quantities lost in each dialysis, estimated by Kopple et al. (1973) to be approximately 6-8 grams of free amino acids and 3-4 grams of bound amino acids, are mostly of non-essential amino acids and largely derived from the intracellular pool as there is little or no depression of plasma amino acids at the end of dialysis (Rubini and Gordon, 1968). The amino acid losses are easily replenished by eating during dialysis and greatly minimised by the use of glucose containing dialysate (Kopple et al., 1973). Thus, providing the diet is adequate, the losses during dialysis would not affect the plasma amino acid profile. If, on the other hand, the intake is poor, these losses would undoubtedly exacerbate protein deficiency and lead to disrupted protein metabolism.

(3) Intestinal absorption

Transport of amino acids from the gut into the circulation after a protein meal could influence the concentration of plasma amino acids. However, in the post absorptive state, the intestine does not appear to play a major role in the regulation of plasma amino

acid composition (Frame, 1958).

Few studies on intestinal absorption in uraemia have been carried out. Gulyassy et al. (1970) reported reduced absorption of tryptophan in uraemic patients and Kassler et al. (1973) found a decrease in arginine absorption in uraemic rats. The extent, however, of the impairment of amino acid absorption from the gut in uraemia is uncertain and the few observations reported do not provide evidence that alterations in intestinal transport could account for the plasma amino acid abnormalities.

(4) Altered renal metabolism

The kidney is involved in the metabolism of a number of amino acids. It is, for example, a major contributor to plasma serine levels in normal individuals. In renal failure, the absence of this function probably accounts for the reduced serine to glycine ratio which is found almost universally.

In normal subjects a linear relationship exists between tyrosine/phenylalanine ratio (T/P) and glomerular filtration rate (Kopple et al., 1972), and a direct relationship between plasma tyrosine levels and the degree of renal impairment has been demonstrated (Giordano et al., 1970). Since there is evidence that renal phenylalanine hydroxylase (the enzyme converting phenylalanine to tyrosine) is reduced in uraemia independent of dietary intake (Wang et al., 1975), a decreased formation of tyrosine by the kidneys may contribute to the altered T/P ratio in chronic renal failure.

(5) Impaired amino acid metabolism

The changes in plasma amino acids may represent a change in amino acid utilization for protein synthesis or energy production, changes in release or uptake by cells, or a combination of these factors.

Skeletal muscle, by virtue of its mass, contains the largest free amino acid pool in the body which is available for protein synthesis, and it is also the tissue which is mainly broken down in conditions of catabolism. Bergstrom et al. (1975) have shown in samples of skeletal muscles from uraemic patients significant increases in the intracellular concentrations of the majority of amino acids except for threonine, valine and tyrosine. These findings suggest either a block in amino acid incorporation into protein, a defect in transport across cell membrane, or increased breakdown in cellular protein. These observations are important because intracellular amino acid concentrations reflect more closely the metabolic state of the patient. Moreover, it has been suggested that imbalances in the intracellular concentrations of the branched-chain amino acids could lead to a decrease in the synthesis rate of cellular protein (Buse and Reid, 1975; Fulks et al., 1975).

(6) Endocrine influences

Changes in balances between protein synthesis and degradation can be influenced by a number of hormones:

(a) Insulin: Insulin is the most important hormone controlling energy homeostasis and it is the principal and perhaps the only hormone regulating both muscle proteolysis and amino acid availability for

protein synthesis; it increases the rate of incorporation of amino acids into protein (Cahill, 1971), decreases that of protein degradation in muscle (Fulks et al., 1975), and decreases the rate of gluconeogenesis (Exton, 1972). In uraemia, several factors prevail which could antagonise these important actions on protein and amino acid metabolism. However, studies of insulin actions in uraemia have so far been almost entirely confined to carbohydrate and fat metabolism.

Old data reported by Mirsky in 1938 showed that administration of insulin to nephrectomised dogs decreased the rate of protein catabolism of muscle and in turn increased the utilisation of exogenous amino acids by skeletal muscle of the uraemic dogs. More recently, Arnold et al. (1977) reported a relative decrease in amino acid uptake by skeletal muscle of uraemic rats in response to insulin administration. Whether the insulin resistance, known to occur in uraemic patients, is as important in nitrogen metabolism as it is in carbohydrate metabolism is not clear.

(b) Other hormones: Changes in circulating levels of glucagon, growth hormone, glucocorticoids and other hormones occur in uraemia. Their possible effects on protein metabolism as related to growth are considered in Chapter 4.

EFFECT OF URAEMIA ON BODY PROTEIN ECONOMY

In normal man subjected to starvation or suffering from protein-energy malnutrition, nitrogen excretion declines in an exponential manner as adaptive processes proceed to conserve body

protein (Cahill and Aoki, 1970; Waterlow and Alleyne, 1971). In contrast, during disease or trauma, with negative calorie balance, the body is much less efficient in preserving its protein (Duke et al., 1970) and significant protein losses during disease were first shown by nitrogen balance studies at the turn of the century by Coleman and Dubois (1915). Changes in nitrogen balance are usually reflections of changes in muscle protein and these in turn correlate with growth in the case of children (Waterlow and Alleyne, 1971).

Protein depletion, particularly muscle protein (Coles, 1972), reduces soluble cell protein content in muscle (Delaporte et al., 1976), negative nitrogen balance and retardation of growth are common findings in uraemia. Moreover, the low-protein diet for treatment of chronic renal failure is often followed by marked muscle cell protein depletion (Campanacci et al., 1970), and some uraemic patients remain in negative nitrogen balance despite an adequate energy and protein intake (Richards, 1975). These observations along with the possibility, discussed above, of increased energy demands for anabolism suggest an impairment in the efficiency of energy utilisation to preserve body protein.

Protein synthesis and degradation

The protein constitution of the body is in a constant state of change with tissue proteins continuously being broken down and resynthesised and a balance is obtained between synthesis and degradation.

The reduced cell mass in uraemia may be a result of failure to sustain an adequate balance between protein synthesis and degradation

(i.e. a relative decrease in synthesis or an increase in degradation, or both). Although no specific catabolic effect of uraemia has been identified, studies in uraemic patients and experimental animals have shown profound abnormalities in protein turn over (Coles, 1970; Shear, 1969; Bianchi et al., 1972; Abittol and Holliday, 1976). However, the possible superimposed effects of malnutrition makes the interpretation of data,particularly those in the clinical studies, difficult and the conclusions reached are rather inconclusive. Coles et al. (1970) found impaired synthesis and degradation of I-labelled serum albumin as well as a shift of albumin from the extravascular to the intravascular space. However, these abnormalities are found in any state of malnutrition (James and Hay, 1968). Studies by Shear (1969) in uraemic rats, using single injection of $^{14}_{C}$-leucine, have shown that net protein synthesis, while increased in lung and heart, was decreased in skeletal muscle. His findings suggested that the source of amino acids for increased protein synthesis in the liver was skeletal muscle. However, similar results were found in rats fed low protein diet (Waterlow and Stephens, 1968). Abittol and Holliday (1976) studied protein synthesis and degradation in uraemic rats and pair-fed controls thereby eliminating the nutritional influences; they found both synthesis and degradation to be reduced in uraemic rats in comparison with pair-fed controls, but the degree of catabolism was more growth limiting in uraemic rats than controls. Bianchi and his group (1972) studied albumin turn over in uraemic patients on low protein diets and demonstrated a marked reduction of the total albumin pool and proportionately reduced catabolic rates. The synthesis rate was also reduced but equal to the catabolic rate. They concluded that a new equilibrium

state in protein turn over had been reset at a level significantly lower than normal. They claim that the abnormalities were not due to the dietary treatment per se as the changes were independent of the duration of the diet (1 month - 5 years). Studying albumin catabolism in the early phases of the development of the uraemic syndrome and during the uraemic state in a group of patients on dialysis therapy, the same group (Mariani et al., 1974) reported a highly increased catabolic rate in the acute phases of uraemia or relapses of it. They made the point that increase in catabolism in uraemia is only transient but is not followed by subsequent compensatory increases in synthesis rate to restore the previous metabolic state as is the case with recovery from non-uraemic catabolic illnesses. These findings, if confirmed, suggest a failure of adaptive increases in synthesis rate to counteract catabolic episodes, such as acute infections and may be relevant to the persistence of protein depletion in chronic renal failure. Indirect evidence of increased muscle catabolism is provided by the finding of increased alanine release from muscle in uraemia (Garber, 1978). Since alanine is the most important gluconeogenic substrate and since substrate supply is the main drive for gluconeogenesis (Exton, 1972), hepatic glucose production may be increased in uraemia. More recently, Holliday et al. (1977) showed that after starvation muscle protein synthesis decreased and degradation rate increased more in moderately uraemic than control rats. This suggests that the catabolic response to nutritional stress is more exaggerated in uraemia.

A fundamentally important question is what factor(s) render(s) the uraemic organism less efficiently adaptive to calorie deficiency and consequently insufficiently anabolic.

In the absence of disease, the organism adapts to calorie deprivation by rapid mobilisation of endogenous fat for energy production so as to minimise amino acid degradation and preserve body protein (Cahill and Aoki, 1970). A number of hormones, particularly insulin, are involved in the control of these adaptive metabolic processes. It may be that in uraemia the protein wasting is caused by a relative lack of substrates other than amino acids to support energy production in muscle. Such a derangement may in turn be related to abnormalities in hormone metabolism arising from non-nutritional causes but as a result of renal failure. Although many hormonal changes have been described in uraemia (discussed in some detail in Chapter 4), their relationship to energy substrates and, therefore, their relevance to body protein economy have not been assessed. The present study is concerned, in part, with such an assessment.

CHAPTER 4.

HORMONAL CHANGES IN URAEMIA WITH PARTICULAR REFERENCE TO GROWTH REGULATING HORMONES.

Despite intensive investigation of the biochemical and metabolic disturbances that accompany renal failure, study of the associated hormonal changes has received only intermittent attention. However, during the past ten years or so, there has been growing interest in the changes which may occur in the function of various endocrine glands in uraemia. This is partly due to advances made in immuno assay techniques and also to the recognition of the role of the kidney in the metabolism of many hormones.

Renal failure may affect hormonal balance in many ways: (1) by increasing or reducing hormone synthesis, (2) by altering hormone binding to plasma proteins or tissue receptors, or (3) by altering hormone metabolism or renal clearance. These effects may be (a) directly due to uraemia per se or reduced functioning renal mass, (b) indirect as a response to specific metabolic disturbances, or (c) related to protein energy malnutrition so commonly associated with the uraemic state. In interpreting published reports of hormonal changes in renal failure particular account has to be taken of population differences, especially in regard to degree of renal insufficiency, nutritional status and treatment differences; and also of possible interference of uraemia in vitro function tests. Certain hormones are now known to be essential for normal growth. Among them are the pituitary growth hormone (GH), insulin, thyroid hormone and the gonadotrophic and sex hormones. Little is known about the

endocrine status of uraemic children, in particular the hormonal changes which may relate to their poor growth. Evidence, already discussed, suggests that the uraemic patient is insufficiently anabolic and since failure of growth may be equated with poor anabolism, this review will be restricted to those hormones known to have anabolic or catabolic functions.

GROWTH HORMONE (GH)

The importance of pituitary growth hormone in the regulation of growth is generally accepted and the subject extensively reviewed (Korner, 1965; Raiti and Blizzard, 1970).

Metabolic actions of GH

Early work established that growth hormone is an anabolic hormone with potent stimulatory action on nitrogen retention. This subsequently proved to be a reflection of its ability to stimulate synthesis of tissue proteins by enhancing amino acid incorporation and, when there is a decrease in the level of substrate amino acids, it also promotes amino acid uptake by cells (Kostyo and Rillema, 1971; Turner et al., 1976). In addition to having this anabolic property, GH has also been found to inhibit glucose utilization and uptake (Daughaday and Kipnis, 1966), and to possess the ability to promote mobilization of fat from storage depots causing FFA release from adipose tissue and enhancing the FFA uptake into muscle (Rabinowitz et al., 1965). The first action is abolished by administration of glucose or food.

GH levels fluctuate enormously during the course of the day. Hunter and Rigal (1966) studied this diurnal variation in children aged 9-15 years and showed very low GH levels in the first two hours after meals, but levels rose to much higher later. Values were consistently high during the night. The authors inferred that GH was secreted at those times when the mobilization of fat and the conservation of amino acids was most necessary, and consequently allows for continuity of growth during short term fasting conditions.

Growth hormone in uraemia

Recognition of abnormal GH metabolism in uraemia resulted from the search for a cause of carbohydrate intolerance of uraemia. Samaan et al. (1966, 1970) were the first to demonstrate elevated fasting GH levels and a paradoxical rise in GH following glucose infusion in non-dialysed and dialysed uraemic patients. Subsequently, high GH levels in uraemia have been observed by others (Horton et al., 1968; Wright et al., 1968; Spitz et al., 1970; Orskov and Christensen, 1971). An exaggerated rise in GH levels following insulin has also been seen in uraemic patients despite a diminished hypoglycaemic response (Kokot and Kuska, 1972). To date, there are no reports of GH measurements in children with renal failure.

The cause of the elevated GH levels in uraemia is not known. It has been variously attributed to impaired degradation (Cameron et al., 1972) or increased secretion (Taylor et al., 1972). The techniques used have involved the administration of labelled or unlabelled human growth hormone and the measurements of either metabolic clearance rate (MCR) or plasma half disappearance time ($t_{\frac{1}{2}}$)

It has been suggested that the metabolic clearance rate of GH is reduced in renal disease (Cameron et al., 1972). However, since the plasma $t_{\frac{1}{2}}$ of labelled GH was not prolonged in nephrectomised rats until significant uraemia developed, Rabkin et al. (1972) proposed that the kidney itself is not important in GH degradation and ascribed the prolonged $t_{\frac{1}{2}}$ of GH to secondary metabolic effects of uraemia. Somatostatin (growth hormone release-inhibiting factor) inhibits GH secretion by direct action on the anterior pituitary. Using intravenous exogenous somatostatin the $t_{\frac{1}{2}}$ of endogenous GH can be calculated and Pimstone et al. (1975) found the disappearance rate of endogenous GH in patients with chronic renal failure to be within normal limits. Their data suggest that high levels of plasma GH found in these patients are caused by hypersecretion rather than impaired clearance. If there is hypersecretion in uraemia, the stimulus for this is unknown. It may relate to protein-energy malnutrition (Heard and Stewart, 1971; Pimstone et al., 1967). Wright et al. (1968) found an inverse correlation between serum GH and serum albumin in 41 non-dialysed uraemic patients and suggested that the high GH levels are a reflection of protein malnutrition, a consequence of renal failure. However, Samaan and Freeman (1970) could not confirm this correlation and Davidson et al. (1976) failed to find any relationship between dietary protein intake and GH levels in adult uraemic patients undergoing maintenance haemodialysis. The range of serum albumin and different therapeutic setting make comparison of the reported data difficult. Furthermore, serum albumin may not be a useful index of nutritional status in uraemic patients. Lunn et al. (1973) have shown that, in children with protein-energy malnutrition

(PEM), plasma GH may not increase until plasma albumin concentration falls to a very low level. Obviously, more sensitive indices of protein malnutrition in uraemia, such as plasma transferrin concentration, will have to be used to establish whether or not protein malnutrition is an underlying cause of the GH abnormality in uraemic patients.

The importance of associated changes in carbohydrate, fat and protein metabolites in uraemia in the pathogenesis of high GH levels is not known, and the stimulus for and the metabolic effects of the increased GH concentration in uraemia remain obscure.

SOMATOMEDIN (SM)

Despite intensive study of the structure and function of growth hormone, the mechanism of growth hormone stimulation is still not well understood. Because of the ineffectiveness of GH in stimulating anabolic processes in cartilage from hypophysectomized rats in vitro and the effectiveness of normal serum, but not hypophysectomized rat serum, in restoring these anabolic processes,Salmon and Daughady (1957) originally proposed what is now termed the somatomedin hypothesis. This states that GH does not stimulate growth of tissues directly but leads to the generation of secondary hormonal factors collectively called somatomedin (SM), which stimulate growth of both extraskeletal and skeletal tissues (Daughaday et al., 1972). Van Wyk et al(1974) characterized SM as a serum factor that (1) stimulates the growth of at least one tissue, (2) is under GH control, (3) has insulin like action and (4) promotes sulphur uptake by cartilage.

Recently, several somatomedins have been purified (Uthne, 1973; Van Wyk et al., 1974) and the factor stimulating the uptake of sulphate into chicken embryonic cartilage has been designated somatomedin A (Uthne, 1973). Hall and Fillipsson (1975) demonstrated a significant correlation between somatomedin A in serum and growth rate in healthy children. Abnormal somatomedin levels are seen in some growth disorders, e.g. they are high in acromegaly and low in pituitary dwarfism, and growth response and serum somatomedin have been shown to be closely correlated in GH treated hypopituitary children (Hall and Olin, 1972).

Serum levels of somatomedin and growth rate may not, however, always parallel the level of growth hormone. Low somatomedin with associated growth failure despite elevated GH was first noted in children with Laron type dwarfism and their growth failure in this disorder has been attributed to defective somatomedin generation secondary to a more generalized lack of responsiveness to GH (Daughaday et al., 1975).

Nutritional status may be another factor that influences the relation between somatomedin and GH. Decreased SM and poor growth despite normal or elevated GH levels have been reported in children with Kwashiorkor-type malnutrition, and when the children were referred, SM levels increased to normal (Grant, 1973). A similar relationship was demonstrated in fasted rats which were subsequently refed (Phillips and Young, 1975).

Growth failure in children with chronic renal insufficiency may be due, in part, to somatomedin deficiency. Saenger et al. (1974) have reported low SM levels by bioassay in uraemic children. These levels rose to normal in those patients who had substantial improvement in creatinine clearance and growth rate following transplantation. More recently, Schwalbe et al. (1977) found a significant correlation between SM levels and linear growth rate in a group of children with various degrees of renal insufficiency. Whether the reported reduced levels of plasma SM activity in uraemia is a direct result of uraemia per se or secondary to protein-energy malnutrition so commonly associated with uraemia is not known.

Although the liver has been suggested as an important site for SM formation and perhaps also the kidney, virtually nothing is known of the mechanism involved in its formation nor of the factors controlling its homeostatic regulation. It is not known whether there is a feed-back mechanism between SM and GH or not. Whether the depressed SM levels are important aetiologically in the growth failure of children with uraemia, or simply a marker of undernutrition is also unknown.

Serum SM activity is at present quantified by bioassays in vitro using the incorporation of radioactive sulphate. Inorganic sulphate concentration may be raised in chronic renal failure and may, therefore, influence the uptake of radio labelled sulphate. Saenger et al. (1974) found that the SM activity of uraemic sera with very high inorganic sulphate concentrations may be underestimated by about 30%. Phillips et al. (1978) confirmed the interference of serum inorganic sulphate in the estimation of bioassayable SM activity in uraemic subjects and further noted an increase in SM activity after

haemodialysis, possibly due to removal of SM inhibitors. Audhya and Gibson (1974), however, found no evidence for changed SM activity in the presence of raised sulphate with serum dilutions less than 40%.

Sufficient quantities of the purified SM are not yet available to study the in vivo effects of SM administration and, therefore, caution must be observed in drawing conclusions concerning the physiologic significance of these substances in vivo.

INSULIN

The effects of chronic renal failure on insulin metabolism and its possible relationship to the observed derangements in the metabolism of carbohydrates, lipids and proteins have already been discussed.

Insulin is a very important anabolic hormone; it has synergistic action with GH in promoting protein synthesis and growth (Manchester and Young,1961), and its effect on cellular growth has been emphasised with the concept that GH is responsible for growth by increasing cell number and insulin by increasing cell size (Cheek et al., 1970). The relationship between insulin and growth is, however, complex because both growth hormone and increased nutrition may result in increased insulin secretion (Curry et al., 1973; Wagner and Scow,1957), and insulin is required for a full anabolic effect of GH (Milman et al., 1951). Inadequate insulin therapy of diabetic children is associated with retardation of growth (Birkbeck, 1972) despite higher than normal GH levels (Hansen and Johansen, 1970). Protein-energy malnutrition is also associated with decreased levels of insulin and impairment of growth despite high GH concentration. Increased food

intake with adequate insulin secretion, on the other hand, may increase growth in the absence of GH; children with craniopharyngioma may become hyperphagic and obese after removal of the tumour, with hyperinsulinaemia and normal or increased growth rate despite low GH levels. When hyperglycaemia is accompanied by increased insulin secretion, increased growth may occur as in infants of diabetic mothers who have hyperplasia of islet tissue, hyperinsulinaemia and increased birth weight and length (Molsted-Pedersen and Jorgensen, 1972).

Although somatomedin shares certain features with insulin, this relationship between insulin and somatomedin is not clear. Because both SM and insulin levels are low in malnourished children while GH is raised (Grant, 1973), it was suggested that insulin may play a permissive role in SM production by the liver but there is no experimental evidence that insulin is involved in SM generation. Recently, Phillips and Young (1976) demonstrated a significant association between insulin deficiency and reduced SM activity and between SM and growth in streptozotocin-induced diabetes in rats. However, the low SM levels may not be causally related to reduced insulin concentration but relate to protein depletion in the diabetic state.

As pointed out in Chapter 3, glucose intolerance is often seen in uraemia but circulating insulin levels are usually high as the degradation rate is abnormal, and tissue resistance to the action of insulin is well established. There is also evidence that specific binding of insulin is markedly reduced (Soman and Felig, 1977). Whether the uraemic effect on insulin metabolism and its peripheral action is important in the pathogenesis of growth failure in uraemic

children is not known. Indeed, it is not yet elucidated that the abnormalities in carbohydrate and insulin metabolism, documented in adult patients, occur in children with chronic renal failure. The state of insulin secretion and its relationship to other growth determinants remain to be investigated in these children.

GLUCAGON

Glucagon and catabolism

The important catabolic effects of glucagon are those related to its actions on the liver and adipose tissue (Unger, 1971). In the liver, it stimulates glycogen breakdown and gluconeogenesis (Park and Exton, 1972). In adipose tissue, it stimulates lipolysis. Further, Marliss et al. (1970) reported that infusion of glucagon in physiological amounts in man caused a marked depression of some plasma amino acid levels and enhanced net urea excretion. This suggested that glucagon increased protein catabolism, perhaps to fuel gluconeogenesis.

Although many factors influence the secretion of glucagon, glucose and amino acids are the most important in the physiologic regulation of pancreatic glucagon secretion (Unger, 1971).

The opposing effects of insulin and glucagon and the various interactions between them in the fed and fasted state lead to the concept that they function as an opposing pair, with insulin acting as an anabolic hormone and glucagon as a catabolic hormone (Unger,1971). Unger and his co-workers proposed that the insulin to glucagon molar ratio, rather than the absolute values of either insulin or glucagon, is the major determinant of fuel homeostasis in normal man; an

increase in the ratio would favour anabolism and a decrease favour catabolism. The implication of this is that glucagon plays an important role of nutrient homeostasis by mobilizing stored energy substrates when exogenous sources are lacking or when there is an increased demand for metabolic fuel.

In recent years, an important role for glucagon in the regulation of normal blood glucose concentration and in the pathogenesis of diabetes this has been postulated (Unger et al., 1970). However, more recent work (Felig et al., 1976) has detracted from these ideas and laid emphasis on the primary role of insulin-deficiency in the diabetogenic actions of glucagon.

Glucagon in uraemia

Glucagon was inevitably investigated as a possible mediator of glucose intolerance in uraemia. Bilbrey et al. (1974) have shown that pancreatic glucagon concentration in patients with chronic renal failure is almost invariably elevated and is correlated directly with plasma creatinine levels. They also demonstrated a normal rise of glucagon after a protein meal but the levels remained inappropriately high during hyperglycaemia induced by glucose infusion. Since haemodialysis improved glucose tolerance in some of their patients without altering the high plasma glucagon levels, the authors concluded that the hyperglucagonaemia was not responsible for the glucose intolerance of uraemia. Sherwin et al. (1976) confirmed the finding of Bilbery et al. and further noted that the hyperglycaemic effect of exogenous glucagon is increased in undialysed uraemic patients and that dialysis normalizes the glycaemic response to

glucagon. On the basis of these findings, they suggested an increased tissue sensitivity to glucagon in uraemia which is correctable by dialysis accounting, therefore, for improved glucose tolerance despite persistent hyperglucagonaemia.

Several investigators have indicated that immunoreactive glucagon (IRG) may exist in several forms in the plasma of normal and diabetic subjects (Weir et al., 1975). Kuku et al. (1976) demonstrated similar heterogeneity of plasma glucagon in patients with chronic renal failure. They showed that the hyperglucagonaemia in these patients is largely due to an increase of fraction (B) of approximately 9100 mol. weight, which is consistent with proglucagon, although fraction (C) (mol. wt.3500), corresponding to the biologically active glucagon, is also considerably elevated.

The cause of elevated plasma glucagon concentration in chronic renal failure is not known. The observation that a rapid rise of plasma glucagon levels can be produced by urethral ligation in dogs, but not by infusions of urea or creatinine (Bilbery et al., 1974), and that plasma glucagon concentration falls to normal levels in patients following successful renal transplantation (Bilbery et al., 1975), lead this group of investigators to believe that hyperglucagonaemia of uraemia is the result of decreased glucagon catabolism as a result of a reduced functioning renal mass. Although the kidney plays a role in pancreatic glucagon catabolism (Lefebvre et al., 1974), the liver is the major site of its degradation (Assan, 1972). Furthermore, it is difficult to separate the effects of uraemia per se and those of reduction in renal tissue in both

Bilbery's clinical and animal studies; the dogs undergoing bilateral ureteral ligation did not, for example, develop sustained hyperglucagonaemia till prior to death from uraemia. Moreover, the infusion of urea or creatinine may not be equated with uraemia as other uraemic 'toxins' or deranged metabolism may be responsible for the hyperglucagonaemia. The fall of plasma glucagon levels following successful transplantation may equally be due to amelioration of uraemic state. Increased secretion, at least of the 3500 mol. weight fraction (active glucagon) is likely since the elevated levels in patients with chronic renal failure is suppressable to normal by glucose infusion (Kuku et al., 1976). In addition, high concentrations of glucagon have frequently been observed in severely catabolic non-uraemic patients (Unger, 1971; Lindsey et al., 1974), and in this respect hyperglucagonaemia of uraemia may be related to derangements in metabolism and other hormonal changes associated with chronic renal failure. Whatever the cause , the significance of the elevated levels of this catabolic hormone on protein conservation and thereby upon growth in uraemic children remains to be evaluated.

CORTISOL

Cortisol and catabolism

Since the observations made by Ingle more than 25 years ago (Ingle, 1951), it has been generally accepted that cortisol is necessary for a catabolic response to occur, but its role is only 'permissive' and that cortisol itself has no true regulatory role in catabolism. However, more detailed studies of metabolism in

the past ten years have delineated the functions and target tissue of glucocorticoids more precisely. Whereas the effects of these compounds on hepatic gluconeogenesis dominated the literature for many years, it is now clear that cortisol exerts a direct effect on a wide variety of peripheral tissues including muscle, adipose tissue, lymphoid tissue and bone (Leung and Munk, 1975). The nature of the response induced by cortisol, however, varies in different tissues. One fairly general effect is inhibition of glucose uptake by cells with simultaneous insulin resistance which may underlie some of the catabolic effects of this hormone (Munk, 1971).

Administration of glucocorticoids results in a marked increase in the free amino acid pool in muscle and in the release of amino acids particularly alanine, the main gluconeogenic amino acid, into the circulation (Wise et al., 1973). The activity of key gluconeogenic enzymes is increased with resultant increase in hepatic glucose production. The release of non-esterified fatty acids from adipose tissue is increased primarily as a result of a decrease in re-esterification and also a depression in the activity of lipogenic enzymes (Diamant and Shafrir, 1975).

Catabolic states such as trauma, burns and infection are associated with increased adrenocortical secretion (Beisel, 1975), and strong correlations suggesting a causal relationship between plasma cortisol levels and various energy substrates have been found during burn injury (Batstone et al., 1976). It is evident from the work of Batstone et al. (1976) and Alberti et al. (1975) that cortisol together with other catabolic hormones play a major role in fuel

mobilization when energy requirements are increased or when the utilization of glucose as energy substrate is defective.

Cortisol and growth

It is now well recognised that inhibition of growth is a prominent problem in children treated chronically even with relatively low doses of corticosteroids. Falliers et al. (1963) have shown that as little as 6 mg of prednisone per square meter per day, corresponding to only 2-3 times average daily secretion of hydrocortisone, results in suppression of somatic growth in children. Moreover, cessation of corticosteroid administration or the cure of spontaneous Cushing's syndrome in children after a period of growth suppression may be followed by a period of accelerated growth rate 'catch up growth' (Prader et al., 1963; Loeb, 1976).

Although the growth-suppressive effects of the corticosteroids are clear, the mechanism(s) of this inhibition is not. One potential mechanism that was considered is an inhibition of growth hormone secretion (Frantz and Rabkin, 1964). However, most of the data in this respect comes from studies on adults and more importantly the argument against this mechanism is the fact that GH administration does not overcome the growth inhibiting effect of corticosteroids (Morris et al., 1968). Interference either with somatomedin generation by the liver or with somatomedin action on growing cartilage has recently been postulated as a possible mechanism (Phillips et al., 1974). The results have not been conclusive and certainly this is an issue that needs further examination.

Loeb (1976) considered the inhibitory effects of glucocorticoids on nuclear DNA synthesis and cellular replication as the basis of corticosteroid-induced growth suppression. This direct cellular effect may be mediated through changes in the hormone receptor complex binding to nuclear chromatin and messenger RNA synthesis (Baxter, 1977).

Adreno-cortical function in uraemia

Little is known about the function of the adrenal cortex in association with chronic renal failure. Fanconi (1954) suggested, without evidence, that adrenal hypertrophy in renal disease with excess circulating corticoids might cause growth retardation. Adrenal hypertrophy involving mainly the zona fasciulata and zona glomerulosa has been found in uraemic rats (Morrison, 1962) and in patients with renal failure (Nibira, 1971). Whether the adrenal hypertrophy is associated with increased corticosteroid production response is not known. Plasma cortisol levels in uraemic adult patients are in the upper limits of normal or raised (Snodgrass et al., 1970; Bacon et al., 1973; Bindeballe et al., 1973; Pichler et al., 1972). There are no values reported for children on dialysis but Betts et al. (1975) found plasma cortisol levels to be within normal range in 11 non-dialysed children with various degrees of renal insufficiency who also exhibited a normal diurnal rhythm. However, only five normal controls were included in this study. Several diurnal patterns of cortisol secretion have been reported; whereas some investigators (Varghese et al., 1969; Snodgrass et al., 1970) found the diurnal rhythm to be abnormal, others (Lindsay et al., 1969; Betts et al., 1975) reported a normal rhythm.

These discrepancies may be due to different methods used to measure plasma cortisol or to heterogeneity of patients studied with the effects of uraemia and dialysis not separated. The cortisol responses to ACTH stimulation and dexomethasone suppression are normal (Pichler et al., 1972; Lindsay et al., 1969; Barbour and Sevier, 1974), but the plasma cortisol half-life is consistently prolonged.

Effect of dialysis

Englert et al. (1958) described a fall in conjugated, but not free, 17-OHCS levels after a single dialysis. Subsequent studies (Asbach et al., 1973; Bindeballe et al., 1973; Pichler et al., 1972) demonstrated specific oscillatory patterns of plasma cortisol levels in response to haemodialysis. Although the patterns reported were variable, some studies suggested early removal of cortisol into the dialysate, followed by a rise in plasma cortisol above normal limits. This increase is ACTH-induced as a consequence of an increase in the negative component of the feed-back mechanism (Hrubesch et al., 1973). The overshoot of feed-back regulation may be due to stress but the exact mechanism is not clear.

Cortisol dialysance is small amounting to only 1-3% of normal daily production (Mishkin et al., 1972), but the metabolic clearance rate increases by 30% during dialysis (Deck et al.,1968). There is also evidence, though not conclusive, that in most dialysed patients the amount and rate of cortisol secretion exceed expected normal values (Mishkin et al., 1972).

The evidence from published data suggests an increase in the level of circulating cortisol in uraemic patients. Since cortisol causes a pronounced reduction in nitrogen retention and suppresses growth even in relatively low doses, it seems probable that such levels of cortisol if sustained may adversely affect growth in uraemic children. Such a possibility has not been evaluated.

THYROID HORMONES

Effects of thyroid hormones on growth and development

The importance of thyroid hormone in normal postnatal growth is well established, and abundant evidence shows that this hormone is the essential stimulus to skeletal maturation throughout childhood, although at puberty, and after, the thyroid role in bone maturation is taken over by the sex hormones. The decreased rate of growth in hypothyroidism is reflected in other measurements of growth; hydroxyproline excretion, which is an index of body growth, is decreased in hypothyroid children and increased after therapy (Graystone and Cheek, 1968); hypothyroid children show a far less positive nitrogen balance than normal for their age or size which could be corrected by thyroid hormone treatment. Cell population studies in children with acquired hypothyroidism (Cheek, 1968) indicate that the reduced body size associated with hypothyroidism results from a decreased cell population.

Although it has been suggested that growth retardation in hypothyroidism may result from a secondary growth hormone deficiency, the evidence from animal studies indicates that thyroid hormone deficiency is the primary cause (Greenberg et al., 1974).

Thyroid hormone excess can also influence growth; linear growth and bone maturation are enhanced in hyperthyroid children (Hung et al., 1962). However, if present in great excess, thyroid hormone can be toxic and impairment of ultimate growth despite accelerated bone maturation has been demonstrated in rats (Best and Duncan, 1969).

The process of sexual maturation is also influenced by the thyroid status; hypothyroidism in childhood generally delays puberty (Ahuja et al., 1969), but occasionally precocious puberty may occur.

Thyroid hormones in uraemia

Patients with chronic renal failure are generally considered to be euthyroid. However, a variety of disturbances in thyroid function indicating both hyperthyroidism (Bailey et al., 1967) and hypothyroidism (Ramirez et al., 1973; Silverberg et al., 1973; Carter et al., 1974) have been described in these patients. Extra thyroidal factors associated with uraemia and its treatment make the interpretation of thyroid function tests difficult.

Alterations in the binding of thyroid hormones to their respective binding protein either as a result of reduction in the plasma concentration of the binding proteins or displacement by a variety of phenols and indols retained in large quantities in the serum of uraemic patients (Bailey et al., 1967) will influence the total and free plasma thyroid hormone levels. Reduced iodide clearance and raised plasma levels of inorganic iodides in uraemia may affect the thyroid uptake of I^{131} (Koutras et al., 1972).

Furthermore, since the kidney plays an important role in the metabolism of thyroxine and its conversion to the more biologically active tri-iodothyronine (Burke et al, 1972) it is possible that impairment of this mechanism occurs in renal disease (Carter et al., 1974). In addition, although the losses of thyroid hormones across haemodialysis membranes are negligible (Silverberg et al., 1973), post-dialysis levels of free thyroxine are significantly higher than pre-dialysis levels because heparin administration decreases the binding of thyroxine to protein (Schatz et al., 1969), but there is a progressive fall in serum thyroxine and free thyroxine index associated with long term haemodialysis (Dandona et al., 1977). Changes in the plasma thyroid hormone levels may, therefore reflect failing renal function or haemodialysis treatment rather than thyroid gland dysfunction. Laboratory indices which suggest hyperthyroidism can often be explained by non-specific extrathyroidal factors but it is more difficult to exclude mild hypothyroidism. Reductions in the serum levels of total T_4, free T_4 and T_3, and decreased T_3 resin uptake have been demonstrated in both dialysed and non-dialysed uraemic patients (Silverberg et al., 1973; Joasoo et al., 1974; Carter et al., 1974; Dandona et al., 1977). The reasons for the low levels of thyroid hormones remain controversial. The thyroxine binding globulin capacity was found to be normal by many investigators (Ramirez et al., 1973; Joasoo et al., 1974; Neuhaus et al., 1975); therefore, the low levels of T_4 and T_3 are unlikely to be due to a decreased concentration of carrier protein. According to Lim et al. (1975) and Carter et al. (1974) a defect in the peripheral conversion of T_4 to T_3 in these patients may explain the low serum levels of

T_3, but this is an unlikely explanation when circulating T_4 levels are decreased as well. It is possible, however, that thyroxine monodeiodonation may be altered to produce a metabolically inactive form of triiodothyronine (rT_3) which does not cross-react in T_3 radioimmunoassay (Burr et al., 1975). The preferential conversion of T_4 to rT_3 rather than to T_3 in uraemia is suggested by the finding of high rT_3 levels in uraemic patients (Chopra et al., 1975).

If hypothyroidism exists in uraemic patients the impairment may be due to reduced TSH output by the pituitary (Silverberg et al., 1973; Czernichow et al., 1976), or to decreased TSH-responsiveness of the thyroid gland (Ramirez et al., 1973). The plasma TSH response to synthetic thyrotrophin-releasing hormone (TRH) is usually found to be blunted (Hasegawa et al., 1975; Czernichow et al., 1976; Dandona et al., 1977). The reason for this abnormal response is not clear; it may be due to decreased sensitivity of the pituitary to TRH or to an abnormal thyrotroph cell function the nature of which is unknown, but raised GH levels have been implicated (Czernichow et al., 1976). However, the TSH response to TRH stimulation is not uniformly subnormal in the different patients studied; a normal or even exaggerated response has been demonstrated in some patients (Ramirez et al., 1973).

Thyroid response to TSH stimulation has been variously described as normal (Ramirez et al., 1973) or diminished (Dandona et al., 1977), but the pituitary-thyroid axis seems to be intact.

In summary, the thyroid status in uraemic patients remains controversial. The evidence from data reported suggest increased

incidence of relatively low levels of circulating thyroid hormones particularly T_3. Regardless of the mechanisms involved, the metabolic significance and particularly the effect upon growth and development in uraemic children of such suboptimal levels of circulating thyroid hormones require to be assessed.

SEX HORMONES

Understanding of the role played by the sex hormones, i.e. androgens, oestrogens and gonadotrophins in growth, particularly at puberty, has advanced considerably in the past decade and is well reviewed (Root, 1973).

Anabolic actions and effects upon linear growth

(a) Androgens:

The relationship of androgens to muscular development in the male is well recognised and it is assumed that it is largely due to protein-anabolic action on skeletal muscle (Kochakian et al., 1956). Androgens stimulate protein-synthesis and produce an increase in the amino acid pool and nitrogen retention (Haak, 1966). Although increased nitrogen retention is not synonymous with accelerated growth, it is certain that advancement in growth cannot occur without it. Many animal and clinical studies have shown clearly that androgens stimulate both linear growth and skeletal maturation. However, osteogenesis and sexual maturation are the main target system for androgens and, therefore, skeletal maturation proceeds more rapidly than linear growth under the influence of androgens. Congenital adrenal hyperplasia with over production of adrenal

androgens produces increased growth rates, accelerated epiphyseal maturation, early puberty and reduced ultimate stature. Similar effects are produced in normal children and in children with 'constitutional short stature' when given androgens (Geller, 1968). With androgen deficiency, as in male hypogonadism, the period of growth is greatly extended with marked delay in the onset of puberty and epiphyseal fusion.

(b) Oestrogens:

Unlike androgens, oestrogens have only a modest anabolic effect. They have a specific growth promoting effect on certain tissues such as the breast and the female genitalia. Their action on bone is similar to that of androgens in accelerating skeletal maturation and hastening epiphyseal closure - with eventual inhibition of linear growth. Although it has been suggested that oestrogens may interfere directly with cartilage growth and peripheral somatomedin action (Herbai, 1970), this has not been substantiated (Phillips et al., 1975). More definite suppressive effects of oestrogens on plasma somatomedin levels both in experimental animals and in man have recently been demonstrated (Phillips et al., 1975; Wiedemann, 1976).

In contrast to oestrogen, the possible influence of androgens on somatomedin metabolism has received no attention, and the inter-relationships between gonadal steroids, growth hormone and somatomedin during periods of growth and sexual maturation remain to be further clarified.

Hormonal control of the onset of puberty

In the normal child there are important and poorly understood central nervous factors which become operative at the age of puberty. The common pathway for central nervous system effects is the hypothalamus and particularly luteinizing hormone releasing factor which is a hypothalamic factor promoting the secretion of FSH and LH. These gonadotrophins act on the gonads and lead to maturation and the secretion of sex steroids. This mechanism is not operative during childhood mainly because of the very sensitive nature of the feed-back mechanism during this period, in which very small amounts of gonadal steroids and perhaps adrenal secretions suppress hypothalamic activities. An intact hypothalamic pituitary-gonadal axis, as well as normally functioning gonads, is important in the process of sexual maturation.

Nutritional status of the individual and the attainment of a certain percentage of their ideal body weight are considered to be important factors in the initiation and the progress of sexual development and clearly, the adolescent growth spurt takes place against the background of genetic, nutritional and environmental factors, but this dramatic increase in growth velocity is best explained by the hormonal changes that accompany the physical manifestations of puberty.

As pointed out above, both androgens and oestrogens accelerate linear growth and in terms of the adolescent growth spurt the all important steroids are the gonadal steroids; testosterone from the testes and oestrogens from the ovary (Root, 1973). The growth spurt

is preceeded by gradual rises, in the male of testosterone and in the female of oestrogens. Whether oestrogen acts directly or whether it is mediated through other factors remains to be determined.

The role of pituitary growth hormone during puberty remains speculative. However, quantitative and qualitative changes in the secretion of GH occur at puberty, and there is evidence that both gonadal steroids and GH are required for pubertal growth spurt, though GH has only a permissive effect (Zachmann and Prader, 1970). This has to be on a background of normal thyroid, insulin and cortisol secretions.

Sex hormones in uraemia

Considerably more information is available regarding sex hormone function in adult uraemic males than in uraemic females, and surprisingly, in view of the delayed pubertal onset, there are no reported observations on uraemic children with the exception of reports of delay in the onset of puberty.

Frequently observed clinical abnormalities in male patients include gynaecomastia, testicular atrophy, azoospermia, impotence and decreased libido. Amenorrhoea, infertility and decreased libido are common findings in female patients.

Low testosterone levels are found in adult males (Chen, 1970;Guevara et al., 1969; Holdsworth et al., 1977) and may be related to some of the clinical findings mentioned above. Testosterone insufficiency may also be partly responsible for the muscle atrophy and anaemia

associated with uraemia (Gupta and Bundschu, 1972). Since the plasma testosterone binding affinity is either normal or increased (Chen, 1970; Gupta and Bundchu, 1972; Hagen, 1976), the low plasma testosterone levels cannot be due to defective protein binding.

Abnormal levels of the gonadotrophic hormones (FSH and LH) are seen. Elevated FSH and LH levels with raised FSH:LH in both males and females have been reported (Hagen et al., 1976; Olgaard et al., 1975; Holdsworth et al., 1977). On the other hand,others have found elevated LH levels but normal FSH values (Stewart-Bently et al., 1974; Distiller et al., 1975).

Studies of hypothalamic-pituitary-testicular inter-relationships suggest a primary testicular defect: (a) FSH and LH responses to luetenizing hormone releasing hormone (LHRH) are normal, (b) the responses of FSH and LH to clomiphene stimulation are usually normal, (c) blunted testosterone response to exogenous gonadotrophin and (d) the suppression of LH by testosterone propionate is usually normal. However, some patients have either high LH levels with normal testosterone, or normal LH levels with low testosterone. Both combinations indicate a derangement in normal feed-back mechanisms, or a primary hypothalamic-pituitary abnormality (Sawin et al., 1973; Guevara et al., 1969). In a more recent study Holdsworth et al. (1977) have shown that the feed-back mechanism is intact and the pituitary function, as assessed by stimulation and suppression tests, is normal. They confirmed a primary testicular failure as the major cause of reduced testosterone levels. Furthermore, they demonstrated that both reduced metabolic clearance

rate and increased production of LH contribute to the raised plasma LH levels.

PROLACTIN

Raised prolactin levels are commonly associated with gonadal dysfunction. In women infertility and menstrual irregularity occur (Bohnet et al., 1976) and in men impotence, loss of libido and galactorrhoea are common clinical findings (Thorner et al., 1977). However, the relevance of hyperprolactinaemia to pubertal delay in children is not yet established (Thorner, 1978 - personal communication).

Prolactin in uraemia

Hyperprolactinaemia occurs in a high percentage of both male and female patients with chronic renal failure (Hagen et al., 1976; Nagel et al., 1973; Olgaard et al., 1975). Its relationship to the gonadal dysfunction in these patients is not known and no correlation was found between plasma prolactin and gynaecomastia in dialysed adult male patients (Nagel et al., 1973; Hagen et al., 1976).

The cause of hyperprolactinaemia of uraemia is not known. It may be a result of hypothalamic-pituitary abnormality, be secondary to stress, or simply related to reduced renal clearance. Irrespective of the cause of the elevated prolactin levels in uraemia, the elucidation of its possible role in the delay of sexual maturation and consequently growth is obviously important as hyperprolactinaemia may now be treated using dopamine agonists such as bromocriptine.

PART II

METHODOLOGY

CHAPTER 5

CLINICAL METHODS

THE MEASUREMENT OF GROWTH

(1) Stature and weight:

After removal of shoes and outer clothing, height was measured by a wall stadiometer (Harpenden) using the technique described by Marshall (1966). Body weight was measured by standard beam scales. Observed heights were plotted against both chronological age and bone age using Tanner's growth charts for normal children (Tanner and Whitehouse, 1976). To calculate the significance in deviations of height from the norm at different ages, height was expressed as standard deviation scores (Tanner et al., 1971) according to the formula: HSDS = $X-\bar{x}/SD$; where X = present height; $\bar{x}$ = mean height for age; SD = standard deviation in height for age. Mean height and standard deviation for age in normal children were obtained from standard tables (Tanner et al., 1966).

(2) Growth velocity (GV) (cm/year):

Growth performance over the period of observation (one year) was calculated and expressed: (a) as standard deviation score for bone age (Tanner et al., 1971). The standard deviation score (GVSDS) was calculated as follows: GVSDS = observed GV - mean GV for age/standard deviation in GV for age in normal children. Growth velocities and standard deviations in normal children were obtained from standard tables for normal British children (Tanner et al., 1966). (b) The stage of puberty was taken into account when assessing growth performance (Marshalland Tanner, 1970): The children were classified

according to their puberty rating as being either (i) Prepubertal; (ii) at a stage of sexual development compatible with the accelerating phase of the adolescent growth spurt; (iii) advanced in puberty and probably in the deccelerating phase of growth after peak height velocity. Using these three categories of puberty rating growth performance was then classified into the following three grades, based on the method described by Marshal's group (Wass et al.,1977):

Grade (I) Very poor: Well below (growth velocity $<$ 2cm/year) 3rd centile, taking puberty into account.

Grade (II) Poor : Below 3rd centile (but growth velocity $>$ 2cm/year), taking puberty into account.

Grade (III) Satisfactory: Within normal limits, i.e. on 3rd centile or above, taking puberty into account.

These grades were used with the sexes combined for further analysis. The chronological and bone ages used in the calculations of height velocity were those at the middle of the period of observation.

(3) Skeletal maturation (bone age):

Bone maturation was assessed from hand and wrist X-rays by an independent observer according to the method of Greulich and Pyle (1959).

(4) Puberty status:

Sexual development was estimated by the method of Tanner (1962).

ASSESSMENT OF BONE DISEASE

Renal osteodystrophy was assessed from hand X-rays by an independent observer. Subperiosteal erosions were looked for and the X-rays were graded as follows:

(0) Normal X-rays

(1) Moderate erosions present

(2) Severe erosions present

THE ASSESSMENT OF NUTRITIONAL STATUS

Nutrition may be defined as the process by which the organism utilises food (McLaren, 1976). Nutritional status is, therefore, the state resulting from the balance between the nutrient intake and the nutrient expenditure. A survey of nutrient intake is thus an integral part of nutritional assessment.

There are many other methods available for assessing nutritional status: anthropometric measurements, biochemical tests and biophysical tests. Although most of these methods are adequate for the detection of gross states of deficiency, they are not so satisfactory in the detection of borderline or incipient deficiencies.

For the purpose of this study nutritional status was assessed by (1) survey of nutrient intake and (2) biochemical indices.

(1) Determination of dietary intake:

The methods available for the determination of food intake have been described by Marr (1971) and Davidson, Passmore and Brock (1972). They are as follows:

(i) Estimation from consumption of groups

(ii) Estimation by recall, either from the immediate past (e.g. 24 hours) or as the usual intake (customary diet)

(iii) Measurements as eaten.

The latter method is generally accepted as the most accurate of the techniques available. The precise weighing method which demands close supervision of the subjects by the observer was considered impractical for long term dietary survey, and the weighed record was chosen for the purpose of this study.

The parents or older children were required to weigh and measure accurately all items of food and fluids taken on three successive days (including one dialysis day in the case of the children on regular haemodialysis) each month, usually a Friday, Saturday or Sunday. A food intake form was provided on which was recorded the time and day of food intake and description and quantity of food and fluids taken. The completed forms were checked with the parents by a dietician before analysis.

Calculation of intakes

The intakes were coded and computerized. The food tables used in the computer programme were those of McCance and Widdowson (1969) supplemented by data from manufacturers. The fatty acid content of the diet was calculated from tables compiled and adapted for use by the Unit for Metabolic Medicine, Guy's Hospital, London. The means of the 3 days intakes of the various nutrients were expressed as a percentage of recommended intakes (RI) for healthy children (Department of Health and Social Security, 1969) for the height-age of the child.

As there is no recommended value for the carbohydrates and fat separate from the recommended total energy, it was assumed that the percentage of energy derived from carbohydrate and fat in the normal diet were 46% and 42% respectively (Francis, 1976).

Evaluation of the method

This was kindly carried out by Miss H. Brown (Unit for Metabolic Medicine, Guy's Hospital, London) by the use of duplicate analysis method: Identical portions of each food taken by the child and recorded in the dietary sheet were pooled, homogenised and dried at 105^{o} for 48-72 hours to constant weight. The dried food was ground, mixed thoroughly, and analysed. Nitrogen was estimated by the Macro Kjeldahl method (Markham, 1960). Protein was calculated as N x 6.25. Gross energy contents (GE) were measured with the ballistic bomb calorimeter (Miller and Payne, 1959) and converted into metabolizable energy (ME) from the following equation:

$$ME/g = (GE/g \times 0.95) - (N\% \times 0.075)$$

Highly significant correlations were found when the values for protein and total energy obtained by analysis were compared to those calculated from the dietary records ($r = 0.9527$ and $r = 0.935$ respectively), Table I.

Table I.

	No. Patients	Energy (KJ) Mean	S.D.	Protein (g) Mean	S.D.
Calculated	9	7589	2642	51,7	20.1
Analysed	9	7364	2495	48.2	15.3
Correl.coeff.(r)		+0.9350		+0.9527	
Significance		< 0.001		< 0.001	

Table I: Comparisons between calculated and analytical values for energy and protein in one day's diet.

(2) Biochemical measurements:

In the last ten years or so there has been a continuing interest in biochemical indices of nutritional status. The search has been directed towards finding an index which aids the identification of undernutrition in situations where anthropometric measurements may be unreliable.

Various plasma proteins have been suggested as indices of protein-energy malnutrition in children. These include the plasma concentrations of albumin, pre-albumin, amino acids, transferrin and complement proteins. In this study plasma albumin, amino acids, transferrin and complement C_3 were used as nutritional markers. Plasma transferrin and C_3 concentrations were found to be sensitive indicators of nutritional status of adult patients with chronic renal failure (Young et al., 1975; Ooi et al., 1972).

CHAPTER 6

ANALYTICAL METHODS

HORMONE ASSAYS

Radioimmunoassay (RIA) - Basic Principals:

RIA is a general method by which the concentration of a wide variety of substances, including peptide and non-peptide hormones can be determined. The procedure is based on the original observation of Benson and Yalow (1960) that low concentrations of antibodies to the antigenic hormone insulin could be detected by their ability to bind radiolabelled insulin. It was found that unknown concentrations of the hormone could be determined by measuring their inhibitory effect on the binding of labelled hormone and antibody. The principal of the assay, which underlies all competitive binding assays, involves competition between labelled and unlabelled substances for a limited number of combining sites on a specific receptor. The binding agent could be a plasma protein, an enzyme, or a tissue receptor site. In RIA the binding agent is antibody and the marker molecule is a radio isotope. A fixed quantity of radiolabelled antigen is added to the unknown amount of antigen in the sample and to this mixture a fixed quantity of antibody is added. The antibody binds both radiolabelled and unlabelled antigen without distinguishing between the two forms with the result that the amount of radiolabelled antigen bound by the antibody bears an inverse relationship to the original concentration of unlabelled antigen in the sample. The separation procedure is designed to separate the bound from the unbound antigen, after which the radioactivity of either fraction can be determined.

The requirement is for complete and rapid separation of the bound antigen from the free. At the time of separation, the assay has reached, or is approaching, equilibrium. Systems which rapidly remove one or both of the reactants, or the product, are preferred because they leave little time for a readjustment of the equilibrium.

Many separation techniques are available. They involve the use of (a) differential migration of bound and free fractions, e.g. chromatoectropheresis, (b) precipitation of bound fraction by either an inorganic salt or organic solvents, (c) solid phase e.g. sephadex, (d) absorption, e.g. coated charcoal, and (e) double antibody. In this method a second antibody is used to precipitate the antibody-antigen complex. The second antibody is induced in an animal against the gamma globulin (mainly IgG) of the animal used to raise the first antibody for the assay (e.g. rabbit anti-guinea pig IgG). The method relies upon the antigenic sites on the IgG of the first antibody being distinct from those involved in its antibody activity.

The assay is a comparative one and, as a rule, unknown and standard solutions of the antigen are assayed simultaneously. The measurements of radioactivity obtained from the standard solutions are used to construct a standard curve and the unknown amount of the antigen in the samples can then be determined by interpolation.

THE MEASUREMENT OF INSULIN (IMMUNOREACTIVE INSULIN - IRI)

Numerous modifications of RIA of insulin have been devised.

All are based on the original principle described by Benson and Yalow (1960), the competition between standard or unknown quantities of insulin and known amounts of radiolabelled insulin for a known amount of insulin antibody. The differences exist in the methods of separation of the free from the antibody-bound insulin. The double antibody method involves the use of a second antibody against the guinea pig gamma globulin which results in the precipitation of the soluble insulin-anti-insulin complex. This can then be separated by filtration (Hales and Randle, 1963), or centrifugation (Morgan and Lazarow, 1963). The main advantage of the centrifugation technique is that a single tube can be used for incubation of the reactants, isolation of the precipitate and final measurement of radioactivity. The latter method (Morgan and Lazarow, 1963) was used in the measurement of plasma insulin concentration in this study.

Reagents:

(1) Phosphate buffer: This contained: 5.708 g disodium hydrogen orthophosphate, 1.334 g potassium dihydrogen orthophosphate, 3.572 g sodium EDTA, and 9.00 g sodium chloride. This was made up to one litre with deionized water and the pH of the buffer was adjusted to 7.4. One hundred mg of sodium azide as a preservative was added to each litre of the buffer.

(2) 0.5% Bovine serum albumin (BSA): 9.00 g BSA was added to each litre of phosphate buffer. This solution was used as a dilutent for all the material used in the assay. Henceforth, the term phosphate buffer will imply phosphate buffer containing 0.5% BSA.

(3) Insulin standards: The standard human insulin was supplied by the Medical Research Council, Mill Hill, London, Code No. 66/304, in a freeze-dried form (3 u/ampoule). This was diluted in phosphate buffer to give a concentration of 30,000 μU/ml. One ml quantities of this solution were stored at -20°C. For use the stock solution was diluted in 10 ml phosphate buffer to give a standard solution of 272 μU/ml, which was subsequently serially diluted to give standards of 136, 68, 34, 17, 8.5, 4.25 μU/ml.

(4) Anti-insulin serum: This was purchased ready prepared from Wellcome Reagents Ltd., Beckenham, Kent, Code No. RD 09/10, and supplied in vials containing 0.5 ml of a 1:1000 dilution of guinea pig serum freeze-dried and contained 0.1% sodium azide as preservative. This was diluted in phosphate buffer to give a dilution of 1:60,000. The anti-serum was prepared freshly for each assay.

(5) I^{125} labelled insulin: This was purchased from the Radiochemical Centre, Amersham, Buckinghamshire, Code No. IM 38 0.1 μg of insulin with an approximate specific activity of 5 μC supplied in a volume of 5 ml phosphate buffer, pH 7.4. On receipt 1 ml was diluted to a total volume of 10 ml with phosphate buffer and 1 ml aliquots of this was stored at -20°C. For use this 1 ml aliquot was made up to 15 ml with phosphate buffer, i.e. a final dilution of 150 fold.

To estimate radiation damage, 0.1 ml of the I^{125} labelled insulin used with each assay was precipitated with 2 ml of 10% trichloroacetic acid (TCA), centrifuged at 2000 rpm for 20 minutes, the supernatant decanted off and the precipitate counted for radioactivity. Less than 90% precipitation indicated radiation

damage or denaturation.

(6) Normal guinea pig serum (NGPS): Purchased from the Wellcome Research Laboratories, Code No. VD 11. This was stored at $4^{o}C$. For use 0.1 ml of the NGPS was added to 39.9 ml phosphate buffer. The solution was stored at $4^{o}C$ for no longer than five days.

(7) Rabbit anti-guinea-pig serum (RAGPS): This was prepared in our laboratory by the injection into New Zealand White rabbits of 2 ml of 50% (V/V) NGPS, 1 ml intraperitoneally and 1 ml subcutaneously to the cervical lymph nodes at the base of the neck. Initially injections were given every ten days. After four such injections, the rabbits were bled from an ear vein and the sera separated.

The serum was titrated by setting up a series of sero standards and adding increasing quantities of RAGPS. An amount (20%) in excess of that required to produce maximal precipitation was used in the assay.

When the rabbits had a high enough titre, injection and bleeding were carried out on alternate weeks. The serum was pooled, titrated and stored at $-20^{o}C$.

Assay procedure:

The assay was carried out in polystyrene rubes (Luckhams Ltd., Burgess Hill, Sussex, Type LP3).

Stage 1: The setting of a competition reaction between standard or plasma insulin and labelled insulin and a known amount of

anti insulin serum. All samples and standards were assayed in duplicates. The volumes of the reagents indicated were added to the tubes in the order shown by means of an automatic sample processor (LKB Ultralab System 2071):

0.6 ml phosphate buffer

0.1 ml standard insulin solution or plasma sample

0.1 ml I^{125} labelled insulin

0.1 ml anti-insulin serum.

The contents of the tubes were thoroughly mixed and then incubated for 72 hours at $4^{o}C$.

Stage 2: The separation of the free from the antibody-bound insulin. To each tube was added:

0.1 ml normal guinea pig serum

0.1 ml rabbit anti-guinea-pig serum (or sufficient to ensure complete precipitation).

The tubes were mixed on vortex and incubated for 24hours at $4^{o}C$.

All the tubes were then centrifuged for 30 minutes at 2000 rpm at $4^{o}C$. The supernatants were carefully decanted and the tubes allowed to drain in an inverted position for ten minutes and then counted in a gamma counter (LKB 80000). The efficiency of the counter assuming 10,000 cpm was $\pm$ 2.5%.

Calculation of results

The individual counts obtained from the stand ard solutions were plotted against the corresponding insulin concentration to construct

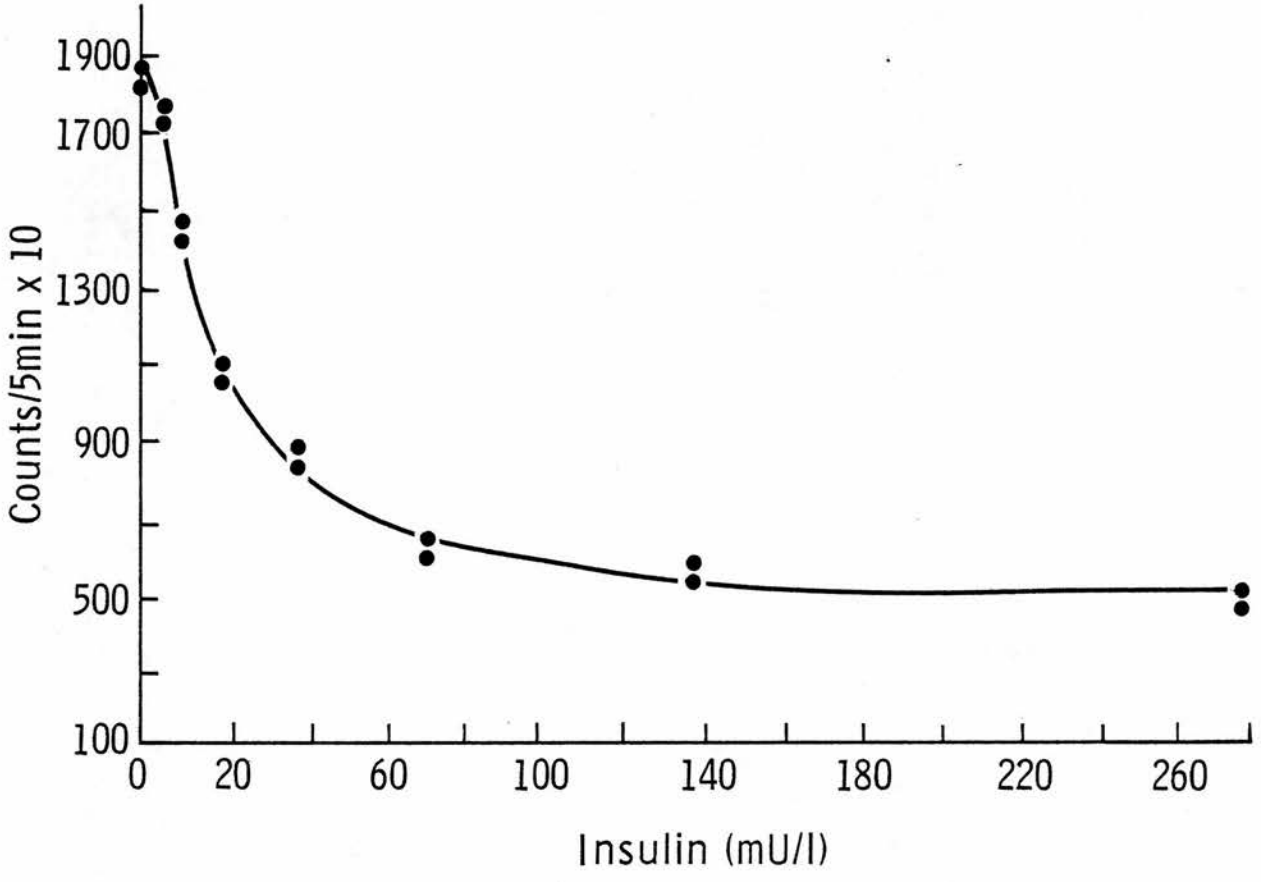

FIG. 1 Typical standard curve for the insulin radioimmunoassay.

a standard curve (figure 1). The means of the duplicate counts for the unknown samples (providing they agreed within ± 2%)were used to read off their insulin concentration values from this curve.

Intra-assay variation

Ten aliquots of a single plasma sample were assayed in a single assay. The mean value was 26.2 mU/l and the standard deviation was 1.2 mU/l (coefficient of variation of 4.6%).

Inter-assay variation

A single plasma sample was assayed on eight occasions. The mean concentration was 24.6 mU/l and the standard deviation was 2.4 mU/l (coefficient of variation, 9.7%).

THE MEASUREMENT OF GROWTH HORMONE (HGH)

The radioimmunological method for insulin assay introduced by Berson and Yalow (1960) was applied to HGH by Hunter and Greenwood (1962). The principal of the method is the same as for insulin outlined above.

The various methods available for RIA of HGH differ mainly in the technique used to separate free from antibody-bound hormone. A number of methods for separation have been published. The double-antibody precipitation method (using a second antibody to precipitate the antibody-bound hormone) was used in this study and was based on the method described by Hartog et al. (1964).

Reagents

(1) Diluent Buffer:

0.05 M phosphate buffer, pH 7.4, containing sodium EDTA, 0.5% bovine serum albumin, sodium chloride and sodium azide, were prepared as described in the insulin assay above. 100 µl of carrier NGPS was added for every 100 ml of buffer used.

(2) I^{125} labelled HGH:

This was kindly supplied by Professor Barbara Clayton, Institute of Child Health, London University. HGH was labelled with radioiodine I^{125}, using the method of Hunter and Greenwood (1962), and had specific activity of 150 - 200 µC/µg. 200 µl of HGH was diluted in 15.8 ml of phosphate buffer, which generally gave 10,000 counts per tube per five minutes, were prepared just before the assay was started.

(3) HGH Standard:

HGH MRC standard A supplied by the Medical Research Council was used. Each ample contained 0.1 mg which was found to contain 0.114 i.u. of HGH by immunoassay against WHO international reference preparation (IRP). Each ample was diluted in 100 ml of phosphate buffer to give a concentration of 1140 µu/ml. 1 ml quantities of this solution were stored at -20°C. For use, the stock solution was diluted in 10 ml phosphate buffer to give a standard solution of 114 µu/ml, which was subsequently serially diluted to give standard solutions of 76, 57, 38, 28.5, 14.25, 7.125 µu/ml.

(4) Anti-HGH Serum:

Guinea pig antiserum to HGH was kindly supplied by the Department

of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital, London. Each batch of anti-HGH serum received was tested by setting up a series of standard curves with various dilutions of the anti-HGH serum. The aim was to obtain about 60% binding of the I-125 labelled HGH in the absence of non-labelled HGH. There was a certain degree of variation from batch to batch but the final dilution of the anti-HGH serum to be used in the assay was between 1:140,000 and 1:150,000.

(5) Rabbit anti-guinea-pig serum (RAGPS):

This was prepared in our laboratory as described above for the insulin assay.

Assay Procedure

Stage I

The setting of a composition reaction between standard or plasma HGH and labelled HGH and a known amount of anti-HGH serum.

All samples and standards were assayed in duplicates. The volumes of the reagents indicated, were added to the assay tubes (polystyrene, Luckhams Ltd.) in the order shown by means of an automatic sample Processor (LKB Ultralab System 2071).

Incubation mixture:

0.6 ml phosphate buffer + NGPS

0.1 ml I^{125} HGH

0.1 ml anti HGH serum.

The mixture was thoroughly mixed and incubated for 72 hours at $4^{o}C$.

Stage 2

The separation of free and antibody-bound HGH.

The tray containing the assay tube was immersed in melting ice.

To each tube was added 0.1 ml of rabbit anti-guinea-pig serum by means of Hamilton microlitre pipette. The contents of the Tubes were thoroughly mixed and then incubated for 24 hours at $4^{o}C$. Thereafter all the tubes were centrifuged for 30 minutes at 2000 r.p.m. at $4^{o}C$. The supernatants were carefully poured off and the tubes allowed to drain in an inverted position for ten minutes. The radioactivity in the precipitates was counted in a gamma scintillation counter (LKB 80000). The efficiency of the counter assuming 10,000 cpm was $\pm$ 2.5%.

The individual counts obtained from the standard solutions were plotted against the corresponding HGH concentrations to construct a standard curve (figure 2). The means of the duplicate counts for the unknown samples (providing they agreedwithin 2%) were used to read off their values from this curve.

Intra-Assay Variation

In order to assess intra-assay variation, ten aliquots of a single plasma sample were assayed in a single assay; the mean value was 12.4 mU/l and the coefficient of variation was 8.2%.

Inter-Assay Variation

To study inter-assay variation, a quality control plasma sample was assayed on ten occasions. The mean concentration was 6.1 mU/l and the coefficient of variation was 12.5%.

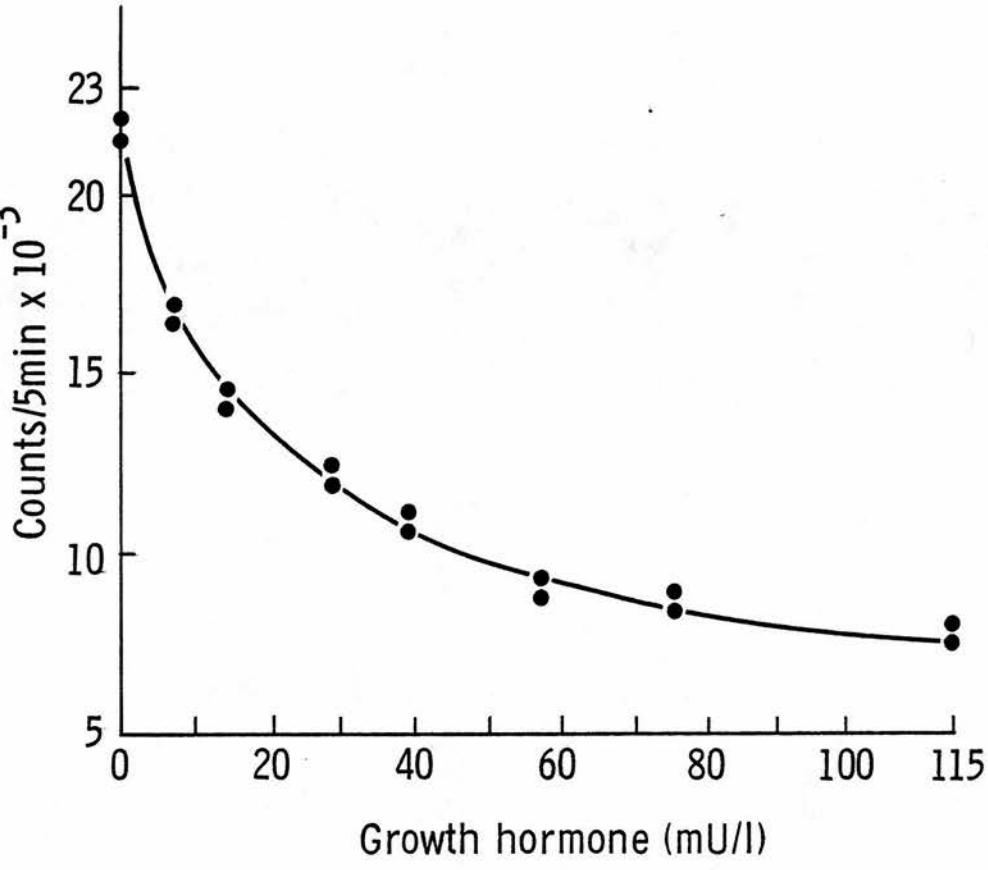

FIG. 2 Typical standard curve for the growth hormone radioimmunoassay.

THE MEASUREMENT OF CORTISOL

Plasma cortisol concentration was measured by a competitive protein binding assay (CPB) using a kit (Cortipac) purchased from the Radiochemical Centre, Amersham, Buckinghamshire, Code No. SC6.

Outline of method

After dilution with distilled water the plasma samples were heated to denature the endogenous cortisol binding protein 'transcortin' and release the cortisol. An aliquot of the diluted denatured plasma sample was mixed with a solution which contains selenium-75 labelled cortisol bound to transcortin and absorbent granules (Sephadex). Cortisol from the plasma sample and the radioactive cortisol compete for binding sites on the transcortin. The free fraction was simultaneously distributed between the granules and the supernatant fluid. At equilibrium, the granules were allowed to settle and the radioactivity of the supernatant was counted. The measured radioactivity was inversely proportional to the concentration of cortisol in the unknown plasma samples. A series of cortisol standards in human serum was treated in an identical manner to the unknown serum samples to produce a standard curve from which values for the unknowns may be interpolated.

Reagents

Each 'Cortipac' kit contained:

(1) 25 test vials; each contained the same weight of absorbent granules and the same volume of buffered transcortin-cortisol (SC^{75}) solution (not more than 0.2 μCi Se^{75} per vial).

(2) 4 vials lyophilised human reference serum containing 2.5, 7.4, 19 and 45 ug/100 ml of cortisol. These were reconstituted just before use by the gentle addition of 500 ul of distilled water to each of the standard serum vials; left to dissolve at room temperature for ten minutes; inverted gently for a few minutes to wash any particles from the cap and left for a further short period for complete solution. The vials were swivelled gently to obtain a homogenous solution.

(3) 25 glass tubes for the heat denaturation stage (see procedure).

Assay procedure

All contents of the kit were stored at $4^{o}C$. They were brought to room temperature just before use. Standard solutions and the unknown plasma samples were assayed in duplicate:

(1) 100 ul aliquot of the serum standard or unknown plasma samples was pipetted into the glass denaturation tubes provided with the kit. 200 µl of distilled water was added to each tube and vortex mixed. The tubes in a rack were then placed in a water bath at $70^{o}C$ for ten minutes.

(2) After allowing the tubes to cool to room temperature, 200 ul of the denatured sample was pipetted into an individual assay vial and the contents mixed continuously at ambient temperature for 45 minutes. A blood cell suspension mixer was used for the purpose.

(3) At the end of the mixing period, the vials were inverted

a few times to wash any granules off the cap and sides and then the granules left to settle for five minutes.

(4) 500 µl aliquots of the supernatant solutions were then transferred into counting tubes. These were counted in a gamma counter (LKB 80000) set for counting selenium-75, for 100 seconds.

(5) Counts obtained from the standard solutions were plotted against their corresponding cortisol concentrations to construct a standard curve from which cortisol values for the unknowns were interpolated.

THE MEASUREMENT OF THYROID HORMONES

THYROXINE (T4)

Serum thyroxine concentration was measured by radioimmunoassay using a commercial kit (T4 RIA (PEG) Kit, Code No. IM 92), purchased from the Radiochemical Centre, Amersham, Buckinghamshire.

Principle: The method depends on the competition between T4 in the serum and I^{125} labelled T4 for binding with T4 specific antibody. The antibody bound hormone was separated by precipitation with polyethylene glycol (PEG). The radioactivity in the precipitate was measured by gamma scintillation counter. The I^{125} labelled T4 bound to antibody was inversely related to the concentration of unlabelled T4 present in the serum.

By measuring the proportion of I^{125} labelled T4 bound in the presence of reference standard sera containing known amounts of T4 a standard curve was constructed and the concentration of T4 present

in the unknown samples was determined by interpolation.

Thyroxine binding proteins (TBG) in the serum may interfere with the serum assay. The degree of interference depends on the concentration of TBG in each serum sample. In this kit, a combination of an appropriate buffer and a TBG blocking agent (Thiomersalate) in the reaction medium has been used to minimise this interference.

Reagents: All reagents required for the assay were provided ready-made in the kit:

1 Vial containing up to 6 uci I^{125} labelled thyroxine.

1 Vial containing anti T4 serum freeze-dried.

4 Vials of reference standards of T4 in human serum (0, 5, 12 and 22 µg T4/100 ml freeze dried)

1 bottle containing 55 ml aqueous polyethylene glycol (PEG)

Reconstitution of reagents

1. Standards: The contents of each of the standard serum vials were gently dissolved, at room temperature, by the addition of 500 µl of distilled water to each vial by means of a micropipette.

2. Antiserum and labelled thyroxine: 10.5 ml distilled water was added to each and mixed until dissolved.

3. Aqueous PEG solution: This was supplied ready for use in a bottle containing 55 ml. The solution was allowed to attain room temperature before use.

Assay Procedure

Standards and samples were all assayed in duplicate. Polystyrene tubes provided in the kit were used in the assay.

1. 50 µl aliquots of the standards and of the unknown samples were pipetted into appropriately marked tubes, by means of micro-pipette (using a new pipette tip for each sample).

2. 200 µl aliquots of the I^{125} thyroxine solution were added to each tube, followed by 200 µl aliquots of the antiserum solution.

3. All tubes were mixed thoroughly on a vortex, and then left to stand for 45 minutes at room temperature.

4. At the end of the incubation period, 1 ml of the aqueous PEG solution was added to each tube and mixed thoroughly on vortex.

5. All tubes were centrifuged for 15 minutes at 2000 rpm at room temperature.

6. The supernatant liquid was decanted and tubes were left inverted to drain for ten minutes.

7. Radioactivity in each precipitate was then counted on a gamma scintillation counter.

8. A standard curve was constructed by plotting the counts obtained from the standard solutions against their corresponding T4 concentration and the means of duplicate counts for the unknown samples were used to read off their values from the standard curve. The normal range of plasma T4 with this assay is 70-190 nmol/L.

TRIIODOTHYRONINE (T3)

T3 was assayed radioimmunologically by T3 radioimmunoassay kit (T3 RIA Kit, Code No. IM 74), purchased from the Radiochemical Centre, Amersham, Buckinghamshire .

The principle of the assay is the same as that described above for the T4 RIA. The main difference were the techniques used for separation of the antibody-bound hormone from the free hormone. In the T3 assay the free T3 was separated by absorption on a solid matrix and the radioactivity of the antibody-bound T3 remaining in solution was measured. Thus, by measuring the proportion of I^{125} labelled T3 bound in the presence of reference standard sera containing known amounts of T3, the concentration of T3 in the unknown samples can be determined by interpolation.

The possible interference by T3 binding proteins in the serum both with the antibody T3 reaction and with the separation of free T3 by absorption on a solid matrix was eliminated in this assay by the combination of an appropriate buffer and a TBG blocking agent. T4 interference is minimized by the use of a highly specific T3 antiserum with a low cross reactivity with T4.

Reagents :

The following freeze dried reagents were provided in the kit:

1 Vial of I^{125} labelled T3 containing not more than 5µci I^{125}.

1 Vial of anti-T3 serum, also containing 100 mg sodium barbitone.

4 Vials of reference standards of T3 in serum, containing 0, 0.7, 2 and 5 mg T3/ml.

1 plastic beaker containing absorbent powder suspension, which contains 200 mg Na barbitone, for the separation of antibody-bound and free T3.

Assay Procedure:

The kit components were stored at $4^{o}C$ and reconstituted just before use. The standards and test samples were all assayed in duplicate. Polystyrene tubes were used.

1. Each serum standard was reconstituted by addition of 500 µl of distilled water by means of a micropipette and gently dissolved.

2. Antiserum and I^{125} labelled T3 were reconstituted by adding 10.5 ml distilled water to each and swirling until dissolved.

3. 50 µl aliquots of the standards and 50 µl aliquots of the samples were pipetted into appropriately marked polystyrene tubes.

4. 200 µl aliquots of the I^{125} labelled T3 solution were added to all the tubes and mixed on vortex.

5. 220 µl of the antiserum solution was then added to all tubes and mixed thoroughly on vortex.

6. The tubes were then incubated in a water bath at $37^{o}C$ for one hour.

7. The separation suspension was reconstituted by adding 55 ml distilled water and stirred by the means of a magnetic bead introduced into the adsorbent suspension.

8. 1 ml of the suspension was added to each tube.

9. All tubes were then capped and placed on a rotator for one hour at room temperature.

10. After allowing the adsorbent to settle completely to the bottom of the tubes, 1.0 ml of the supernate of each tube was pipetted into a counting tube and radio-activity measured by a gamma counter.

11. A standard curve was constructed by plotting the means of the duplicate counts obtained from the standard solutions against their corresponding T_3 concentration. The means of the duplicate counts for the unknown samples were used to read off their values from the standard curve. The normal plasma T_3 range with this assay is 1.2 - 3.0 nmol/L.

THYROTROPHIN (TSH)

A radioimmunoassay kit (HTSH-RIA, Diagnostic Kit), purchased from Abbott Laboratories, Chicago, U.S.A. was used for the determination of the concentration of thyroid stimulating hormone in serum.

Principle:

This was a competitive radioimmunoassay in which non-radioactive Human Thyroid Stimulating Hormone (HTSH) in serum competed with a constant amount of I^{125} labelled HTSH for binding sites on a limited amount of HTSH antibody. The percentage of radioactive HTSH bound was inversely proportional to the concentration of HTSH in the serum. The antibody-bound HTSH (both radioactive and non-radioactive) was separated by precipitation with polyethylene glycol (PEG) from the free HTSH. The radio-activity of the precipitate was then measured by a gamma scintillation counter. The concentration of HTSH in the serum was determined by comparison with reference standard solutions containing known amounts of HTSH.

Reagents

All reagents used in the assay were provided ready made in the kit, and include the following:

1. 1 vial (5 ml) I^{125} HTSH solution, 0.1 M TRIS buffer, 0.3% Bovine Albumin. Activity, 0.1 μ ci/ml, 0.2% sodium Azide as a preservative.

2. 1 vial (6 ml) HTSH standard, 0.0 μ iu/ml HTSH in equine serum. Preservative: 0.2% sodium azide.

3. 5 vials (1 ml each) HTSH standards, concentrations of HTSH: 2.5, 5.0, 10.0, 20.0 and 40.0 μ iu/ml in equine serum. Preservative: 0.2% sodium azide.

4. 1 vial (15 ml) HTSH Antiserum (Rabbit), 0.1 M TRIS buffer, 0.3% bovine albumin, 0.2% sodium azide.

5. 1 vial (2.5 ml) TRIS buffer, 0.1 M TRIS hydroxymethyl-aminomethane, 0.3% bovine albumin, Preservative: 0.2% sodium azide.

6. 1 bottle (150 ml) Polyethylene Glycol (PEG), 16% solution in 0.09 M Barbital buffer.

Procedure

All reagents were kept at $4^{\circ}C$ until just before use when they were brought to room temperature. Standards and unknowns were all measured in duplicates and only polystyrene tubes provided with the Kit were used for the test:

0.1 ml of HTSH of each standard solution and 0.1 ml of the unknown samples were pipetted into appropriately marked tubes.

0.3 ml HTSH Antiserum was then added to each tube and mixed by shaking. The tubes were covered with parafilm and incubated in a water bath at 37°C for three hours. After incubation 0.1 ml I^{125} HTSH solution was added to each tube, mixed gently and reincubated in water bath at 37°C overnight (approximately 18 hours). After the overnight incubation, 3 ml of Polyethylene Glycol solution was added to each tube over a period of less than five minutes and the contents of the tubes mixed thoroughly on vortex. Tubes were then centrifuged at 1000 r.p.m. at room temperature for ten minutes. The supernatant solution was then decanted and the radio-activity in the remaining precipitate counted on a gamma counter. Two tubes containing aliquots of 0.1 ml of I^{125} HTSH solution only were also counted for the determination of total radio-activity (TC).

Calculation of results

The percent bound for each standard or unknown samples was calculated as follows:

$$\% \text{ Bound} = \frac{\text{precipitate cpm}}{\text{mean total count pm}} \times 100$$

A standard curve was plotted on linear graph paper using the mean of % Bound values on the Y axis and various concentrations of HTSH standard on the X axis. Using the six points, the best fit smooth curve was constructed. The mean % bound for each unknown sample was used to read off its TSH concentration from the standard

curve. This assay gives a normal range for serum TSH concentration of 0 - 5 mU/L.

T3 UPTAKE TEST

This was carried out by the use of a kit (Thyopac-3) purchased from The Radiochemical Centre,(Amersham).

Each kit consisted of 12 test vials containing adsorbent granules suspended in buffer containing I-125 labelled T3. A vial of standard reference serum was also provided. The standard serum as a dessicated pooled human serum, which upon reconstitution has a known T3 uptake value which has been accurately determined by the manufacturers.

Test Procedure

1. After allowing the components of the kit to attain room temperature, the dessicated standard serum was carefully reconstituted by the addition of 1 ml of distilled water to the standard serum vial by means of a micro-litre pipette, a homogenous solution was then obtained by gentle shaking of the vial.

2. The test sera were brought to room temperature and 0.1 ml of each of the unknown serum and of the reconstituted standard reference serum was then transferred to a "thypac-test" vial by means of an automatic pipette (Eppendorf). The standard serum was examined in duplicate.

3. All the test vials were then attached to a mixer and mixed at room temperature for 20 minutes.

4. At the end of the mixing period, the contents of the vials were allowed to settle, 1 ml aliquot of the supernatant solution was transferred from each vial by microlitre pipette to a polystyrene tube and counted in a gamma scintillation counter.

Calculation of results

The T3 uptake value of the standard reference serum was known. The T3 uptake value for each unknown serum was calculated by the following formula and expressed in terms of the reference standard:

$$\text{T3 uptake value of unknown serum} = \frac{\text{Countrate of unknown serum}}{\text{Countrate of standard serum}} \times \text{T3 uptake value of standard serum}$$

The normal range for this kit is 92 - 117.

Free thyroxine index (FTI) was calculated from plasma total T4 concentration and T3-uptake value as follows:

$$\text{FTI} = \frac{\text{T4}}{\text{T3 uptake}} \times 100$$

The normal range using T4 RIA (PEG) kit and Thypac-3 kit is 70 - 180.

ASSAYS FOR OTHER HORMONES

(i) Glucagon

Plasma immunoreactive glucagon was measured by radioimmunoassay with a pancreatic glucagon specific (C-terminal reacting) antiserum (Bloom 1974). These measurements were kindly carried out by

Dr. S.R. Bloom in the Department of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital, London.

(ii) Sex Hormones

Plasma testosterone was measured by radioimmunoassay after extraction. An antibody raised by immunisation with a 3-oxime derivative of testosterone conjugated with serum albumin was used (Green et al., 1977).

Plasma luteinizing hormone (LH), follicular stimulating hormone (FSH) and prolactin (PRL) were measured by specific double antibody radioimmunoassays, using MRC standards 68/40 for LH and 69/104 for FSH (Mortimer et al., 1973), and 71/222 for PRL (McNeilly et al., 1973). These assays were kindly undertaken by Dr. L. Rees, Department of Endocrinology, St. Bartholomew's Hospital, London.

(iii) Somatomedin

Serum somatomedin activity was estimated by a bioassay using 11 day old embryonic chick pelvic cartilage and radiolabelled sodium sulphate (S-35) as an index of sulphate incorporation. The method used was essentially that described by Hall (1970). Four cartilage leaflets were used for each of five serum dilutions (1, 2.5, 5, 7.5, 10% v/v) and observations were made in duplicate, giving 8 cartilages for each serum concentration. In each experiment, pooled serum from healthy men, with assigned somatomedin activity of 1.0 units/ml, was used as a reference standard. Radioactivity was expressed per mg dry weight of the cartilage. Liquid

scintillation spectrometry was used to count the radioactivity. Serum somatomedin potency relative to reference serum and tests for parallelism and significant regression were computed using methods described by Finney (1964). The assay was kindly carried out by T. Leaky, The Academic Centre, The London Hospital, London. Statistical analyses were performed by E. Steven, Computer Centre, The London Hospital, London.

PLASMA AMINO ACIDS

Amino acids were measured by ion-exchange chromatography on an automatic amino acid analyser (LKB 3201). A three sodium buffer step system was used with a constant operating temperature of 60°C. These measurements were undertaken by my research colleauge, Dr. R. Counahan, Department of Paediatrics, Guy's Hospital, London. The coefficients of variation of replicate analyses of amino acid standard solutions at the beginning and end of the project and of plasma are shown in Table I.

PLASMA PROTEINS

(i) Albumin: - by autoanalyser (Technicon, bromocresol dye-binding method).

(ii) Transferrin: - by single radial immunodiffusion (Hyland plate).

(iii) Complement protein C3: - by radial immunodiffusion into agar plate containing monospecific antiserum against C3. The results were expressed as a percentage of reference standard serum (Pooled serum from healthy subjects).

TABLE I

	Amino acid standard (n = 9)	Amino acid standard (n = 4)	Plasma (n = 3)
Valine	7.8	2.3	2.6
Leucine	2.6	1.0	1.5
Isoleucine	2.8	3.9	1.6
Methionine	2.1	2.6	-
Phenylalanine	5.5	2.7	0.9
Lysine	2.9	2.7	7.0
Histidine	3.0	7.8	4.5
Glycine	3.5	2.8	5.8
Alanine	3.7	2.8	1.1
Tyrosine	1.2	2.7	4.2

Table I: Coefficients of variation (100 x standard deviation of mean/mean) of replicate analysis of amino acid standard solutions at beginning and end of project and of plasma.

PLASMALIPIDS

Plasma triglycerides were measured by a semiautomated fluorimetric method (Levine and Zak, 1964), plasma non-esterified fat acids (NEFA) by a semi automated fluorimetric method (Carruthers and Young, 1973), serum glycerol by enzymatic method (Eggstein and Krautes, 1966). Lipoprotein electrophoresis was performed on agarose gel (Noble, 1968). These measurements were performed by the Unit for Human Metabolism, Guy's Hospital, London.

METHODS OF OTHER BIOCHEMICAL MEASUREMENTS

Plasma urea: - by autoanalyser (Technicon, diacetylmonoxide reaction).

Plasma Creatinine: - by autoanalyser (Technicon, method 11b, Jaffe reaction).

Blood glucose: - by autoanalyser (Technicon, Ferricyanide method).

Plasma calcium: - by autoanalyser (Technicon, cresolphthalin complexone method).

Plasma phosphate: - by autoanalyser (Technicon, phosphomolybdic acid method).

Plasma bicarbonate: - by autoanalyser (Technicon, phenolphthalein indicator method).

Plasma alkaline phosphatase: - by autoanalyser (Technicon, Phenyl sodium phosphate hydrolysis -phenol - method).

These measurements were performed by the Department of Clinical Chemistry, Guy's Hospital, London.

PART III

STUDIES ON CHILDREN WITH CHRONIC RENAL FAILURE TREATED BY REGULAR HAEMODIALYSIS

CHAPTER 7

PROSPECTIVE OBSERVATIONS ON GROWTH, HORMONAL CHANGES AND NUTRITIONAL AND METABOLIC STATUS IN CHILDREN ON HAEMODIALYSIS.

STUDY I

This study was designed to monitor longitudinally, growth and nutrition as well as a series of growth-regulating hormones, measurements of carbohydrate, lipid and protein metabolism, and other biochemical and haematological parameters in a group of children with end stage renal failure treated by regular haemodialysis in order (a) to establish to what extent the metabolic and hormonal disturbances reported in uraemic adults are manifested in children (b) to determine interrelationships that might exist amongst the nutritional, metabolic and hormonal parameters measured, and (c) to attempt to identify the influence of various nutritional, endocrine and metabolic factors upon growth velocity during the period of observation.

Patients

Sixteen children, nine boys and seven girls aged between 11 - 17 years who had been on regular haemodialysis in the home for more than six months (seven months to six and one-half years) were the subjects of this study. The primary renal disease and clinical data of the patients at the beginning of the study are shown in Table I. None had the nephrotic syndrome, clinical or biochemical evidence of diabetes mellitus or any other systemic disease apart from cystinosis in two children.

Dialysis information

All patients dialyse at home for ten hours overnight on three nights of each week, thirteen using Meltec multipoint dialysers, two using Watson-Marlow Kiil type dialysers and one using a Travenol Ultra-Flo dialyser. The surface area of the kidney was varied according to the child's weight and each dialyser was rebuilt once a week. The dialysates had a glucose concentration of 200 mg/dl and calcium concentration of 2.7 meq/l. Access to the circulation was gained using external Silicone-PTFE Scribner shunts in four patients and subcutaneous arterio-venous fistulae in the remainder. The amount of dialysis received per week was expressed as a product of the dialyser surface area and the total of dialysis hours per week. This was expressed for each child as m^2/hours/kg. body weight, using the child's mean post-dialysis weight during one month at the middle of the assessment period.

Diet

Free diets were allowed with only sodium and potassium restriction where necessary. Fluid intake was limited to approximately 300 - 500 ml per day. Each child also took a variable amount of a highly concentrated energy supplement. This consisted of a milk shake containing egg, double cream (4 cal/ml), milk and a glucose polymer (caloreen, Milner Scientific and Medical Research Co., Liverpool) with an average length of five glucose units which contains 17 kj/g. All of the children took iron, folic acid and vitamin B preparations and some were on dihydrotachysterol (Table 27).

TABLE 1

Patient No.	Sex	Chronological Age (yrs.)	Bone Age (yrs.)	Primary Renal Disease	Access to Circulation	Dialyser Surface Area (m^2)	Amount of dialysis (m^2/hrs/kg)	Duration of dialysis (yrs.)
1	F	16.7	14.5	Reflux nephropathy	Fistula	1.00	0.73	3.5
2	M	15.5	12.0	Focal glomerulo-sclerosis	Fistula	0.77	0.57	2.0
3	F	11.9	12.5	Reflux nephropathy	Fistula	0.77	0.52	1.0
4	M	15.0	14.0	Chronic glomerulo-nephritis	Fistula	0.77	0.54	1.5
5	M	11.8	11.5	Focal glomerulo-sclerosis	Fistula	0.77	0.77	1.0
6	F	12.3	11.5	Juvenile nephro-nopththisis	Fistula	0.77	0.76	2.5
7	M	11.0	8	Reflux nephropathy	Shunt	0.6	0.67	5.5
8	M	14.1	10	Obstructive uropathy	Shunt	0.75	0.46	1.5
9	F	12.3	8.5	Reflux nephropathy	Shunt	0.6	0.80	3.0
10	M	14.1	10	Chronic glomerulo-nephritis	Fistula	0.77	0.71	6.5
11	M	15.1	12.5	Single dysplastic kidney	Fistula	0.77	0.58	1.5
12	F	10.8	10.0	Reflux nephropathy	Fistula	0.6	0.72	0.58
13	M	14.0	10.5	Cystinosis	Fistula	0.77	0.76	1.0
14	M	11.7	10.5	Cystinosis	Fistula	0.77	0.73	2.0
15	F	14.9	14.5	Juvenile nephro-nophthisis	Shunt	0.77	0.45	0.75
16	F	13.6	8	Focal glomerulo-sclerosis	Fistula	0.77	0.59	3.0

TABLE 1. Clinical data of the patients.

Study protocol

Patients were included in the study after successful establishment of regular haemodialysis for a period of not less than six months, and each patient was observed for a period of one year.

All patients were studied at two monthly intervals as outpatients on a predialysis day approximately 30 - 36 hours postdialysis and after an overnight fast for more than twelve hours. Anthropometric measurements were taken at each visit; bone maturation and the degree of osteodystrophy determined at the start, middle and end of period of observation. Evaluation of puberty status was undertaken at the beginning and end of the year of study. In addition, the mean post-dialysis body weight during one month at the start, middle and end of the period was calculated.

Fasting venous blood samples were taken at each visit for the estimations of: urea, creatinine, bicarbonate, potassium,calcium, phosphate, alkaline phosphatase, albumin, haematocrit and haemoglobin. The means of the values thus obtained throughout the study were taken for further analysis. On three occasions, corresponding with the start, middle and end of the period of observation, fasting venous blood samples were taken while the patients were resting and in stable state for simultaneous measurements of the following hormones and metabolic parameters:

(I) Hormones: growth hormone (GH), insulin (IRI), cortisol, thyroxin (T4), triiodothyronine (T3), thyrotrophin (TSH) and T3 Resin Uptake. As puberty rating was performed at the beginning and

end of the study, plasma sex hormones - testosterone, luteinizing hormone (LH), follicular stimulating hormone (FSH) and prolactin (PRL) concentrations were determined on these two occasions only and assessed in relation to puberty status. Serum somatomedin activity (SM) was measured once at the middle of the period.

Thyroid function was further assessed by the evaluation of the integrity of the hypothalamic-pituitary axis by administering thyrotrophin releasing hormone (TRH) to six patients: each received 200 μg of synthetic TRH intravenously with venous blood taken for TSH estimations prior to administration and at 20 and 60 minutes. The values obtained were compared to the responses of seven normal children being investigated for short stature for which no organic cause was ultimately found.

Luteinizing-Hormone-Releasing Hormone (LH-RH) Stimulation Test:-

The hypothalamic-pituitary-testicular function was further assessed in five boys - three prepubertal, one in early puberty and one pubertal - by the determination of the gonadotrophin response to LH-RH stimulation. 100 μg synthetic LH-RH (Hoechst) was administered intravenously. Blood samples were collected prior to the injection and 20 and 60 minutes thereafter for LH and FSH estimations. Values were compared to age-matched control group of six boys being investigated for short stature which ultimately proved to be familial.

The Circadian rhythm of cortisol secretion was evaluated in fifteen patients by the determination of early morning and late evening plasma cortisol levels. The blood samples for hormone analysis were

separated immediately and plasma or serum stored at -20°C until assayed.

(II) Metabolic Parameters:

Plasma amino acids, transferrin, triglycerides (TG), cholesterol (CHOL), nonesterified fatty acids (NEFA), serum glycerol, whole blood glucose (G) and plasma lipoprotein electrophoresis were determined. Aliquots of plasma for amino acids were promptly deproteinized with 1% picric acid, to which norleucine had been added as an internal standard and stored at -20°C prior to analysis. Plasma samples for TG, CHOL, NEFA and lipoprotein electrophoresis were kept at 4°C and analytical procedures carried out within 48 hours. Blood glucose concentrations were measured on the same day. Aliquots of serum for glycerol estimations were stored at -20°C until assayed.

Nutrient intakes were assessed monthly and for the purpose of this study both the mean of the intakes over the whole period of study and intakes recorded within two weeks of blood sampling at the middle of the period were used for statistical analysis.

The means of the various hormones and metabolic indices were measured in each patient over the year and, as growth performance was assessed in relation to bone age determined at the middle of the period of observation, the values obtained at that time were both used for the statistical analysis. A summary of study protocol is shown in Table 2.

TABLE 2.

INVESTIGATIONS	ONE YEAR STUDY: START	MIDDLE	END
Anthropometric Measurements	←	—	→
Post-Dialysis Weight	+	+	+
Skeletal Maturation	+	+	+
Puberty Rating	+		+
Nutrient Intakes	←	—	→
Bone Disease	+	+	+
Hormone Assays :			
Growth Hormone	+	+	+
Insulin	+	+	+
Cortisol	+	+	+
Thyroxine	+	+	
Triiodothyronine	+	+	+
T_3 Uptake	+	+	+
Thyrotrophin		+	
TRH - Stimulation Test		+	
Testosterone	+		+
LH	+		+
FSH	+		+
LH - RH Stimulation Test		+	
Prolactin	+	+	+
Somatomedin		+	
Metabolic Measurements:			
Triglycerides	+	+	+
Cholesterol	+	+	+
Fatty Acids	+	+	+
Glycerol	+	+	+
Lipoprotein Electrophoresis		+	
Blood Glucose	+	+	+
Amino Acids	+	+	+
Transferrin	+	+	+
C_3		+	
Routine Biochemistry and Haematology	←	—	→

TABLE 2. Summary of Study Protocol.

CONTROLS

Eighteen healthy British children aged 12.3 ± 1.9 (1 S.D.) years with normal renal functions were tested as controls. Their clinical data is presented in Table 3. All were asymptomatic and on an adequate diet prior to blood sampling but nutrient intakes were not formally assessed. Nine were being investigated for short stature for which no organic or socio-economic cause was ultimately found; eight were fully recovered from minor surgery and tested shortly before discharge from hospital; one was a laboratory technician.

Fasting plasma amino acids were also measured in a further thirteen healthy children undergoing dental treatment in hospital, and plasma amino acid comparisons were, therefore, made with a total combined control group of 31 children.

Laboratory normal values for plasma sex hormone levels obtained from a large population of boys and girls in various stages of puberty, using the same assays, were used for comparison.

TABLE 3.

No.	Sex	Age (yrs)	Weight (kg)	Height (cm)	Diagnosis
1	F	10	28.5	124	Familial short stature
2	M	10.5	25.8	123	" " "
3	M	12	28	127	" " "
4	M	14	30.5	138	" " "
5	F	13	22	136	" " "
6	F	12.5	27.5	134	" " "
7	M	14.6	40.5	142	" " "
8	M	12	28.5	132.6	" " "
9	M	14	35.5	150	" " "
10	M	10	30.4	140	Inguinal hernia
11	M	11.4	38.5	148	Circumcision
12	F	11.5	35.4	146.8	Fracture of radius
13	F	10.1	31	146	Dental treatment
14	M	12.4	31.5	141	Minor facial injuries
15	M	10.2	29.5	136	Circumcision
16	F	17	51	156.5	Laboratory technician
17	M	13.5	41	153	Dental treatment
18	M	13.1	38	149	" "

TABLE 3. Clinical data of control subjects.

RESULTS

Clinical data of the patients and dialysis information are shown in Table 1. Table 4 depicts the mean ($\pm$ 1 S.D.) of the predialysis plasma urea, creatinine, potassium, bicarbonate, calcium, phosphate and alkaline phosphatase concentrations for each patient during the year of study. Mean haemoglobin, packed cell volume and diastolic blood pressure measurements are also shown.

Haematological and biochemical parameters measured in samples obtained at the middle of the period with simultaneous measurements or other metabolic and hormonal variables are presented in Table 5.

It is apparent from Table 4 that all patients maintained moderately raised plasma urea concentrations and relatively high plasma creatinine levels. Plasma calcium concentration was slightly increased in most of the patients while plasma phosphate concentration was predominantly within normal range with the exception of patients No. 3, 5, 8 and 11 who had moderately elevated levels. Plasma alkaline phosphatase was variably increased in 12 of the 16 patients. Plasma potassium concentration was within normal limits except in patient No. 11 in whom it was increased. There was no evidence of metabolic acidosis as judged by the plasma total bicarbonate concentration ($<$ 20 mmol/l) except perhaps in patients No. 7, 8 and 11 in mid-period sampling (Table 5).

Anaemia was present in all patients but hypertension was not encountered during the period of the study.

Table 6 shows the correlation coefficients obtained between dialysis (amount and duration) and the mean biochemical and haematological

TABLE 4

	HAEMOGLOBIN g/dl (S.D.)	PCV % (S.D.)	UREA mmol/l (S.D.)	CREATININE umol/l (S.D.)	POTASSIUM mmol/l (S.D.)	BICARBONATE mmol/l (S.D.)	CALCIUM mmol/l (S.D.)	PHOSPHATE mmol/l (S.D.)	ALKALINE PHOSPHATASE u/l (S.D.)	DIASTOLIC BP mm Hg (S.D.)
NORMAL RANGE	13.5-14.5	38-42	2.5-7.5	50-130	3.4-5.0	20-32	2.1-2.6	0.8-1.5	7-106	
PATIENT No.										
1	6.1 (0.24)	19.1 (0.78)	12.7 (1.8)	595.5 (52.2)	4.3 (0.32)	23.5 (2.1)	2.63 (0.05)	1.36 (0.16)	144 (50.8)	72 (10.9)
2	7.4 (0.6)	22.2 (0.6)	12.3 (2.7)	612.6 (67.9)	4.7 (0.5)	25.6 (1.3)	2.84 (0.1)	1.08 (0.29)	90.8 (22.1)	82.5 (9.5)
3	8.4 (1.7)	25.4 (5.4)	21.8 (4.5)	668.8 (158.2)	4.3 (0.5)	21.6 (1.7)	2.7 (0.25)	1.77 (0.36)	243.7 (106.8)	70 (16.3)
4	8.1 (1.2)	23.6 (3.6)	16.3 (3.9)	765.7 (144.2)	4.9 (0.4)	23 (1.6)	2.73 (0.16)	1.26 (0.12)	149.9 (40.5)	70.7 (15.4)
5	6.6 (1.4)	19.4 (3.7)	21.2 (7)	594 (140.2)	3.9 (0.47)	23.6 (2.1)	2.7 (0.36)	1.78 (0.94)	56.8 (16.8)	86.6 (9.8)
6	7.4 (1.2)	21.7 (3.6)	10.5 (3.5)	539.4 (153.7)	3.4 (0.6)	24.1 (4.6)	2.61 (0.05)	1.06 (0.35)	180.5 (63)	77.5 (9.5)
7	6.07 (0.42)	17.4 (2.4)	8.7 (8.9)	395.5 (3.6)	4.27 (1.2)	25.5 (4.3)	2.74 (0.08)	1.02 (0.44)	209.4 (68.2)	78.7 (10.3)
8	6.9 (0.4)	20.9 (1.8)	17.5 (5.1)	636.4 (35.1)	4.9 (0.38)	19.9 (4.5)	2.46 (0.07)	1.77 (0.22)	845.3 (322.5)	65 (5.7)
9	5.6 (0.4)	17 (0.7)	10.3 (1.9)	469 (50.7)	3.3 (0.3)	24.8 (1.7)	2.58 (0.07)	1.19 (0.35)	124.2 (17.8)	78 (8.3)
10	6.8 (0.8)	20.6 (2.6)	10.8 (4.3)	522.5 (113)	4.5 (1.3)	24.6 (3.9)	2.73 (0.08)	1.22 (0.97)	369.2 (97.5)	65.8 (14.9)
11	5.9 (1.4)	18.3 (4.4)	21.04 (6.2)	670 (165)	5.7 (0.9)	21 (2.7)	2.62 (0.1)	2.07 (0.64)	86.2 (62.8)	87.8 (20.7)
12	6.1 (0.54)	17.6 (0.98)	14.3 (7.1)	688.2 (148)	3.8 (1.5)	25 (3.1)	2.68 (0.09)	1.28 (0.26)	90.8 (12.7)	76.6 (7.6)
13	6.9 (0.33)	20.6 (0.98)	12.2 (3.7)	478 (166.9)	4.56 (0.26)	24.3 (2.4)	2.7 (0.05)	1.26 (0.55)	203.5 (76.2)	83.3 (13.6)
14	5.95 (0.2)	17.3 (0.95)	10.5 (4.6)	546.8 (78.8)	3.6 (0.5)	27.7 (3.0)	2.9 (0.1)	1.26 (0.32)	116.3 (44.2)	66.4 (12)
15	6.01 (0.66)	17.8 (1.7)	17.5 (7.6)	688 (147)	4.3 (0.74)	21.1 (2.7)	2.76 (0.15)	0.78 (0.09)	23.3 (281)	75 (9.1)
16	6.1 (0.85)	18.3 (2.6)	12.06 (4.9)	575.2 (79.7)	3.75 (0.25)	25.6 (1.9)	2.61 (0.07)	0.92 (0.17)	204.6 (60.2)	86.3 (13.7)

TABLE 4. Mean ($\pm$ 1 S.D.) pre-dialysis biochemical and haematological data of the patients during the year of study.

S.D. = standard deviation.

TABLE 5

	HAEMOGLOBIN g/dl	PCV %	UREA mmol/l	CREATININE µmol/l	POTASSIUM mmol/l	BICARBONATE mmol/l	CALCIUM mmol/l	PHOSPHATE mmol/l	ALKALINE PHOSPHATE u/l
NORMAL RANGE	13.5-14.5	38-42	2.5-7.5	50-130	3.4-5.0	20-32	2.1-2.6	0.8-1.5	7-106
PATIENT No. 1	5.9	19.8	11.8	510	4.4	23	2.65	1.63	27.8
2	7.8	22.9	11.9	589.6	5.1	25	2.82	0.78	71
3	9.4	27.4	23.2	546	4.3	22	2.82	1.44	241
4	7.1	21.4	13.9	589.6	4.5	24	2.77	1.28	78
5	6.1	17.5	17.3	457.6	3.9	24	2.9	1.76	43
6	7.4	20.9	7.6	413.6	3.2	23	2.62	1.18	128
7	6.1	18.8	20.2	800.8	5.9	19	2.7	1.54	227
8	6.4	18.2	13.3	633.6	4.9	18	2.4	1.57	695
9	5.5	17.1	8.6	404.8	3.0	26	2.57	0.86	149
10	6.7	19.4	10.3	457.6	3.6	24	2.77	1.08	319
11	4	13.3	30.5	774.4	6.1	19	2.55	2.85	568
12	5.9	17.6	8.1	607.2	3.7	21	2.6	1.15	78
13	7.0	20.5	8.3	457.6	4.3	27	2.6	2.17	128
14	6	17.8	8.6	457.2	3.4	32	2.87	1.37	92
15	6.4	19.4	8.6	572	5.3	26	2.87	0.74	121
16	6.3	18.2	12.3	536.8	4.1	27	2.65	0.74	192

TABLE 5. Pre-dialysis haematological and biochemical data of the patients measured at the middle of the period of study.

TABLE 6

	Amount of Dialysis $m^2/hr/ug$	Duration of Dialysis (years)
Urea	-0.5079*	-0.6266***
Creatinine	-0.6189**	-0.6231***
Potassium	-0.5704**	-0.0831
Calcium	0.1271	0.0289
Phosphate	-0.0884	-0.3086
Bicarbonate	+0.6297***	+0.3693
HB	-0.3773	-0.1960
PCV	-0.3557	-0.1819
Alkaline Phosphatase	-0.4649	+0.1169

* $P < 0.05$

** $P < 0.02$

*** $P < 0.02$

TABLE 6: Correlation coefficients (r) obtained between amount and duration of dialysis and mean biochemical data.

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data during the year of study. The degree of significance, if any, is indicated. Significant negative correlations were found between dialysis and plasma urea, creatinine and potassium concentrations, and significant positive correlation was present between the amount of dialysis and plasma bicarbonate concentration.

NUTRITIONAL STATUS

(i) Nutrient intakes

Tables 7 and 8 show the mean intakes of total energy, protein, fat and carbohydrates for each of the patients, expressed both in absolute amounts and as percentage of the recommended intake (RI) for normal children of the same height age (DHSS, 1969).

The mean intakes for each patient over the year of study (Table 7) as well as the intakes recorded within two weeks of the blood sampling at the middle of the period (Table 8) are presented. Both methods of assessment showed considerable variation in intake from patient to patient, but relative to body size, of which height age is an approximation, intakes of the majority of the patients were normal. Only two (numbers 8 and 10) had intakes of total energy less than 80% RI and three (numbers 8, 10 and 14) had protein intake less than 80% RI. There were no dietary records available within two weeks of mid-period sampling for patient number 6 but her mean intakes during the year of study showed an intake of 101.4% RI for total energy and 97% RI for protein.

(ii) Biochemical indices of nutritional state

The mean ($\pm$ 1 S.D.) plasma transferrin and albumin concentrations determined at the start, middle and end of period of study and the plasma C3 concentrationsmeasured at the middle of the period are shown in Table 9 and compared to values obtained for normal controls. Individual data for patients are recorded in Table 1 in appendix (A). Because comparison between the three sets of data within the group of patients by means of paired 't' test (Table 10) revealed no significant differences, it was decided to use the values obtained at the middle of the period as

TABLE 7

No.	ENERGY		PROTEIN		CARBOHYDRATE		FAT	
	K Joules/ Day (S.D.)	% RI	g/Day (S.D.)	% RI	g/Day (S.D.)	% RI	g/Day (S.D.)	% RI
1	1029.5 (877.7)	103	59.5 (6.3)	102.6	298.9 (32.5)	113.4	107.9 (9.6)	100.6
2	12698.8 (1467)	120.9	75.5 (8.9)	119.8	375.7 (56.3)	130.6	141.9 (31.9)	121.8
3	10023 (1488)	104.4	87.1 (21)	150.2	209.6 (46.5)	79.2	133.5 (24)	124.4
4	15420 (2746)	131.8	98.5 (19.7)	140.7	350.3 (70.3)	108.8	211.6 (39.8)	162.1
5	9405 (1885)	89.5	77.9 (16.3)	123.6	251.9 (76.4)	87.6	105.8 (24.2)	90.9
6	9732.7 (1020)	101.4	56.3	97	286.6 (79.9)	108.4	110 (1.7)	102.5
7	11455 (3615)	109.1	74 (14.1)	117.4	321	92.5	116	124.8
8	6228 (528.3)	59.3	44.4 (7.2)	70.5	175.8 (26.3)	63	69.8 (12.5)	59.9
9	9454 (1678)	107.4	56.5 (13.7)	106.6	255.3 (46.9)	105.7	115.3 (24.6)	118.4
10	5422 (864.4)	49.2	37.8 (9.5)	60	162 (41.9)	51.6	56.3 (15.5)	48.3
11	15053 (5134)	128.6	96.2 (44)	137.4	394	168.4	196	188.7
12	10511 (4067)	119.4	72.5 (31.7)	136.8	285.5 (116.4)	118.2	123 (54.9)	125.7
13	9255.5 (446)	106.5	54.9 (9.7)	103.6	244 (25.4)	99.9	119.6 (9.2)	122.3
14	3320 (2309)	94.5	32.2 (14.3)	60.7	281.3 (70.3)	116.5	87.1 (38.9)	89.1
15	9979.1 (958.4)	103.4	64.6 (11.1)	111.4	277.1 (51.6)	101	119.4 (17.7)	111.3
16	9251 (1470)	96.3	53.5 (7.4)	92.7	205.9 (35.3)	77.8	131.2 (37.2)	122.3

TABLE 7. Mean ($\pm$ 1 S.D.) dietary intakes of the patients during the year of study.

RI = recommended intake.

TABLE 8

No.	ENERGY		PROTEIN		CARBOHYDRATE		FAT	
	K Joules/ Day	% RI	g/Day	% RI	g/Day	% RI	g/Day	% RI
1	9520	99	60	103	303	101	97	114
2	11698	112	79	125	327	101	135	145
3	12127	126	132	228	244	82	156	184
4	12001	102	66	88	265	80	173	166
5	10983	105	77	122	246	102	150	161
6	-	-	-	-	-	-	-	-
7	14102	134	84	133	378	109	171	184
8	6415	61	46	73	133	56	71	76
9	10964	125	56	106	321	118	128	162
10	4886	47	46	73	136	42	51	55
11	18684	159	128	70	389	107	268	258
12	15911	181	118	183	396	132	198	251
13	9175	104	55	104	237	87	116	147
14	8010	91	18	34	302	111	76	96
15	10306	107	68	117	297	109	116	136
16	12493	130	64	110	389	130	215	253

TABLE 8. Dietary intakes of the patients recorded at the middle of the period of study.

TABLE 9

	Albumin (g/l)			Transferrin (g/l)			Complement C_3 (% RNS)		
	S (SD)	M (SD)	E (SD)	S (SD)	M (SD)	E (SD)	S (SD)	M (SD)	E (SD)
Patients	41.4 (3.4)	42 (4.8)	41.7 (3.9)	1.93 (0.65)	2.41 (0.91)	2.20 (0.48)	-	102.4 (17.3)	-
Controls	43 (1.9)			2.46 (0.52)			118.8(14.1)		
Significance of difference (P)	NS	NS	NS	NS	NS	NS	-	NS	-

TABLE 9. Mean ($\pm$ 1 S.D.) plasma albumin, transferrin and C_3 concentrations in patients and controls. S - start; M - middle; E - end of period of study. RNS - Reference normal serum. SD - standard deviation.

TABLE 10

	S vs M		S vs E		M vs E	
	t	p	t	p	t	p
Albumin	-0.54	NS	-0.48	NS	-0.04	NS
Transferrin	-1.4	NS	-0.82	NS	+0.40	NS

TABLE 10: Significance of variation in plasma albumin and transferrin concentrations determined at the start (S), middle (M) and end (E) of period of study (paired 't' test).
NS = not significant.

well as the mean values over the year of study for further analysis.

The mean and range of plasma transferrin levels in the controls were similar to that reported in the literature when the plasma transferrin level was measured by the radial immunodiffusion method (Wardle et al., 1975). Although there were considerable interpatient variations in plasma transferrin concentration, the mean value in the patients as a group was not significantly different from mean control value. Only one patient (number 9) had a level 2 S.D. below the normal mean. Plasma albumin concentration and protein complement C3 levels were also not significantly different from those of healthy subjects. In individual patients no significant relationships were found between transferrin, albumin and C3, and there was no relation between these plasma proteins and dietary intakes of either total energy or protein. The relationship between the nutritional markers albumin, transferrin and C3 and other variables will be discussed below.

TABLE 11

	Insulin (mU/l)			Growth Hormone (mU/l)			Cortisol (nmol/l)		
	S (SD)	M (SD)	E (SD)	S (SD)	M (SD)	E (SD)	S (SD)	M (SD)	E (SD)
Patients	19.5 (6.6)	20.7 (8.7)	16.8 (4.8)	14.4 (7.2)	13.1 (9.3)	16.1 (8.5)	519.7 (154.3)	425.7 (123.8)	415.6 (138)
Controls	8.4 (5.2)			6.6 (3.2)			355.3 (121.1)		
Significance of difference (P)	<0.001	<0.001	<0.001	< 0.001	<0.01	<0.001	< 0.005	NS	NS

TABLE 11. Mean ($\pm$ 1SD) Plasma Insulin, Growth Hormone and Cortisol concentrations in patients and controls.
S - start; M - middle; E - end of period of study.
SD - Standard Deviation.

METABOLIC HORMONES (insulin, growth hormone and cortisol).

Table 11 shows the patients mean values ($\pm$ 1 S.D.) of plasma insulin, growth hormone and cortisol levels as determined at the start, middle and end of the year of study and compared to normal values obtained from healthy subjects. Statistical analysis by paired 't' test (Table 12) revealed no significant changes in the metabolic hormone concentrations during the period of observation in the patients as a group. Individual data for patients are presented in Table 2 of appendix (A).

TABLE 12

	S vs M		S vs E		M vs E	
	t	p	t	p	t	p
Insulin	-0.618	NS	+1.07	NS	+1.92	<0.1
Growth Hormone	+0.371	NS	-1.158	NS	-1.48	NS
Cortisol	+1.824	<0.1	+1.463	NS	+0.256	NS

TABLE 12: Significance of variation in plasma insulin, growth hormone and cortisol levels determined at the start (S), middle (M) and end (E) of period of study (paired 't' test).
NS = not significant.

Mean plasma insulin levels were significantly higher than normal controls at the first and second sampling and, although they tended to decrease at the end of period of observation, the levels remained significantly

elevated (Table 11). Plasma growth hormone concentrations were significantly raised in patients compared with controls (Table 11) throughout the year with a further increase at the end of the period coinciding with a decrease in plasma insulin. Individual plasma cortisol levels were within the upper range of normal throughout the year, nevertheless the mean concentration at the start of study was significantly raised compared with controls (Table 11) and the average value over the year was significantly increased (Figure 1). Plasma cortisol levels tended to decrease at the end of the period as compared with the initial concentration, but the difference was not statistically significant. Because of the insignificant changes in the pattern of the three hormones, the values obtained at the centre of the period of study and the means of the three samples were considered to represent the basal values and were used as appropriate for further analysis.

(1) Insulin (IRI)

Mean basal plasma immunoreactive insulin was 20.7 ± 8.7 mu/l as determined at the middle of the period and 18.5 ± 5.3 mu/l when three samples for a given patient were averaged. These values were significantly higher than the normal mean of 8.4 ± 5.2 mu/l ($p < 0.001$ and $p < 0.001$ respectively), and do not differ from each other significantly. The average values did not relate to the mean plasma concentration of either urea or creatinine, although in dialysed patients these parameters do not reflect accurately the degree of uraemia. The efficiency of dialysis is difficult to assess, however, the duration of dialysis and the amount of dialysis calculated for each subject, as described in methods, had no apparent influence on plasma insulin concentration.

Relationship between insulin and other metabolic hormones

There was no correlation between plasma insulin and the simultaneously measured plasma growth hormone or cortisol concentrations.

Reports in the literature (Kim et al., 1969) indicated that parathormone (PTH) enhances insulin secretion and that hyperparathyroidism in patients with chronic renal failure on chronic haemodialysis is associated with increased insulin response to glycaemic stimuli (Lindall et al., 1971). The role of PTH could be related to its well known role in controlling serum calcium as insulin secretion is highly dependant on calcium ion in the Islets of Langerhan. Unfortunately plasma PTH concentration was not measured in the patients of the present study, but comparison of the plasma insulin levels in patients with signs of secondary hyperparathyroidism, as judged by X-rays, showed no significant difference. Furthermore, no significant correlation between plasma insulin and calcium concentration could be demonstrated.

Plasma Insulin and Diet

Plasma insulin concentrations did not relate to dietary intakes of total energy, carbohydrate, fat and protein recorded closest to the time of blood sampling and expressed as percentage of recommended intake for height age.

Plasma Insulin and Nutritional Status

Plasma concentrations of transferrin, albumin and protein complement C3, measured simultaneously, as indices of nutritional status, bore no significant relationship to the plasma insulin concentration.

The relationship between plasma insulin and plasma amino acids and other metabolic variables will be discussed below.

(2) Plasma Growth Hormone (GH)

The mean ($\pm$ 1 S.D.) basal plasma GH concentration in the control group was 6.6 $\pm$ 3.2. Nine of the sixteen patients (56.3%) had basal plasma GH levels more than 2 S.D. above this value (Table 2, Appendix A). The mean basal GH concentrations in the patients, determined at the middle of the period as well as the average over the year of study, were significantly higher than normal ($p < 0.01$ and $p < 0.001$ respectively) (Table 11). Plasma growth hormone concentrations did not correlate with the duration of dialysis, the amount of dialysis or plasma urea or creatinine concentrations. Nor was there any relationship between plasma GH and plasma insulin or cortisol concentrations.

Plasma GH and Nutrition

No significant correlation was seen between basal GH levels and either total energy, protein, carbohydrate or fat intakes recorded within two weeks of sampling. Similarly, GH did not correlate with the indices of protein status; plasma albumin, transferrin and C3.

(3) Plasma Cortisol

Figure 1 shows the morning plasma cortisol levels in the patients and controls. Although the cortisol concentrations in the patients were within the reported normal range for children (Barnes et al., 1972), the mean $\pm$ 1 S.D. was 435.1 $\pm$ 109 nmol/l for the patients and 355.3 $\pm$ 121 nmol/l for the controls. This difference was statistically

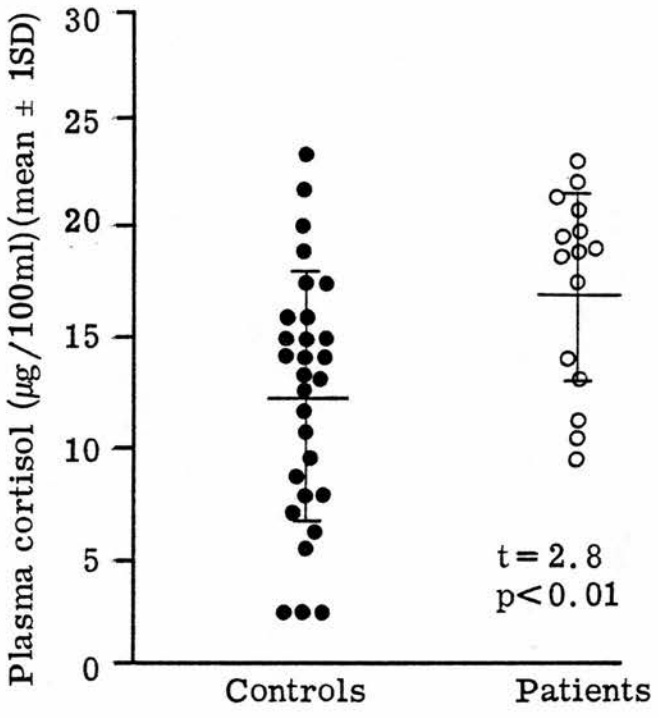

Fig. 1. Plasma cortisol concentration in patients and controls. Horizontal lines represent the mean ± 1 SD.

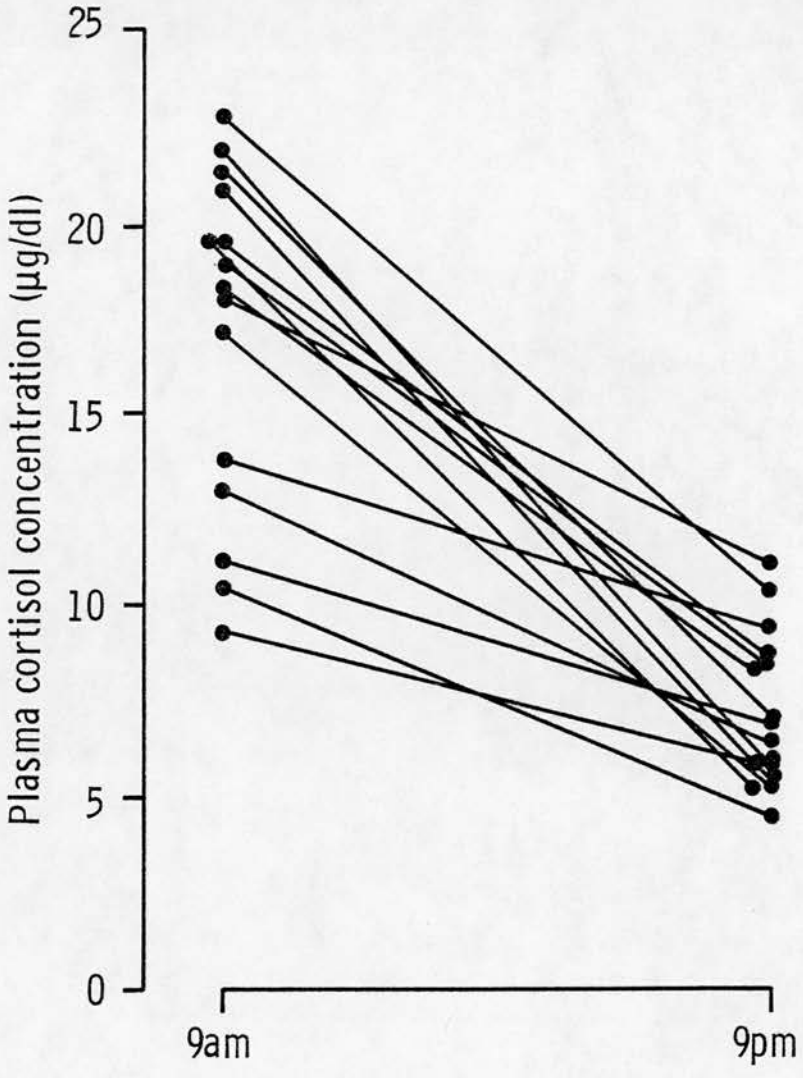

Fig. 2. Morning and evening plasma cortisol levels in patients.

significant ($t = 2.043$, $p < 0.05$). Figure 2 shows in fifteen of the sixteen patients a well marked diurnal variation of plasma cortisol suggesting an intact hypothalamic-pituitary-adrenal axis.

There was a tendency towards higher cortisol levels when basal insulin concentrations were elevated. Those patients with plasma insulin levels exceeding 20 mu/l (> 2 S.D. above the mean for controls) had significantly ($p < 0.02$) higher plasma cortisol concentrations (Figure 3). There was no demonstrable relationship between plasma cortisol levels and GH concentrations, the nutritional status of the patients, the amount of dialysis or the degree of uraemia as assessed by plasma urea and creatinine concentrations.

Serum Somatomedin (SM)

Results for the patients' serum somatomedin activity are shown in Table 13. In view of the possible increase in inorganic sulphate concentration in uraemic sera which may interfere with the bioassay giving falsely low serum somatomedin activity (Phillips et al., 1978) and because no allowance for increased sulphate concentration in the patients of the present study was made, the results were not compared to values obtained from normal controls.

Assuming that sulphate inhibition was the same in all the patients, correlations were tested between serum somatomedin activity and plasma growth hormone, albumin, transferrin and C3 concentrations, and between serum SM and dietary intake of energy and protein. A significant correlation was found only between SM and plasma transferrin concentration ($r = + 0.617$, $p < 0.02$) (Figure 4).

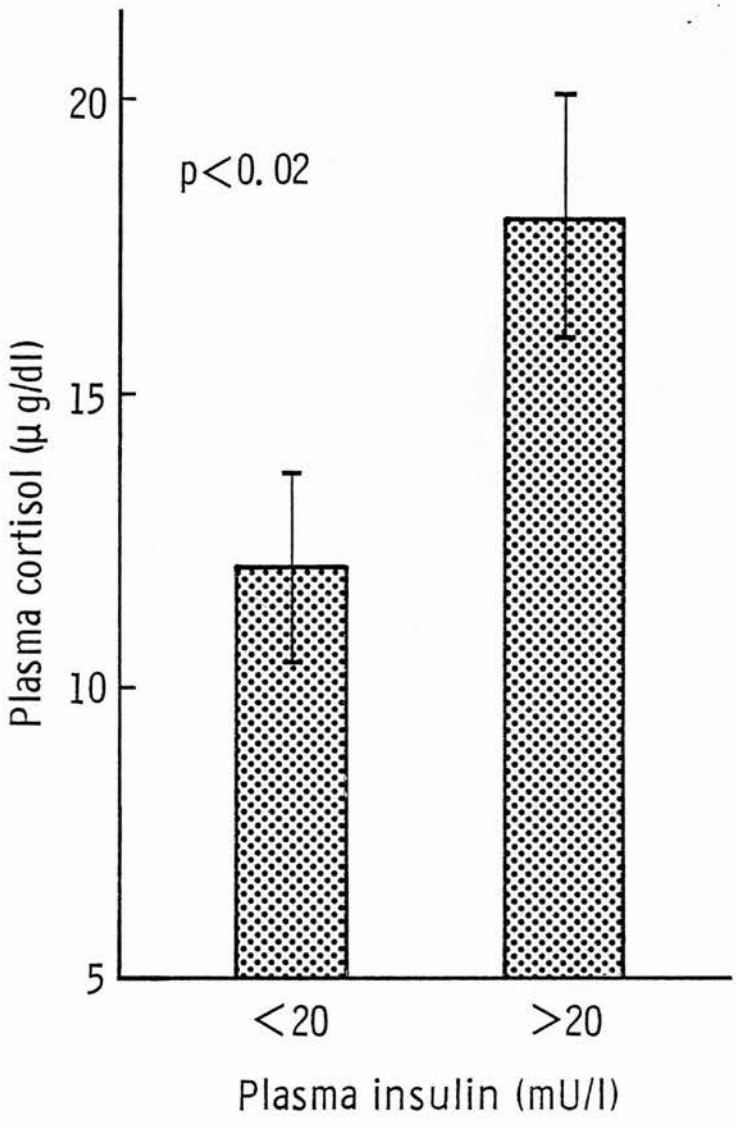

Fig. 3 Relationship between plasma cortisol and insulin levels. Values are the means with their standard errors.

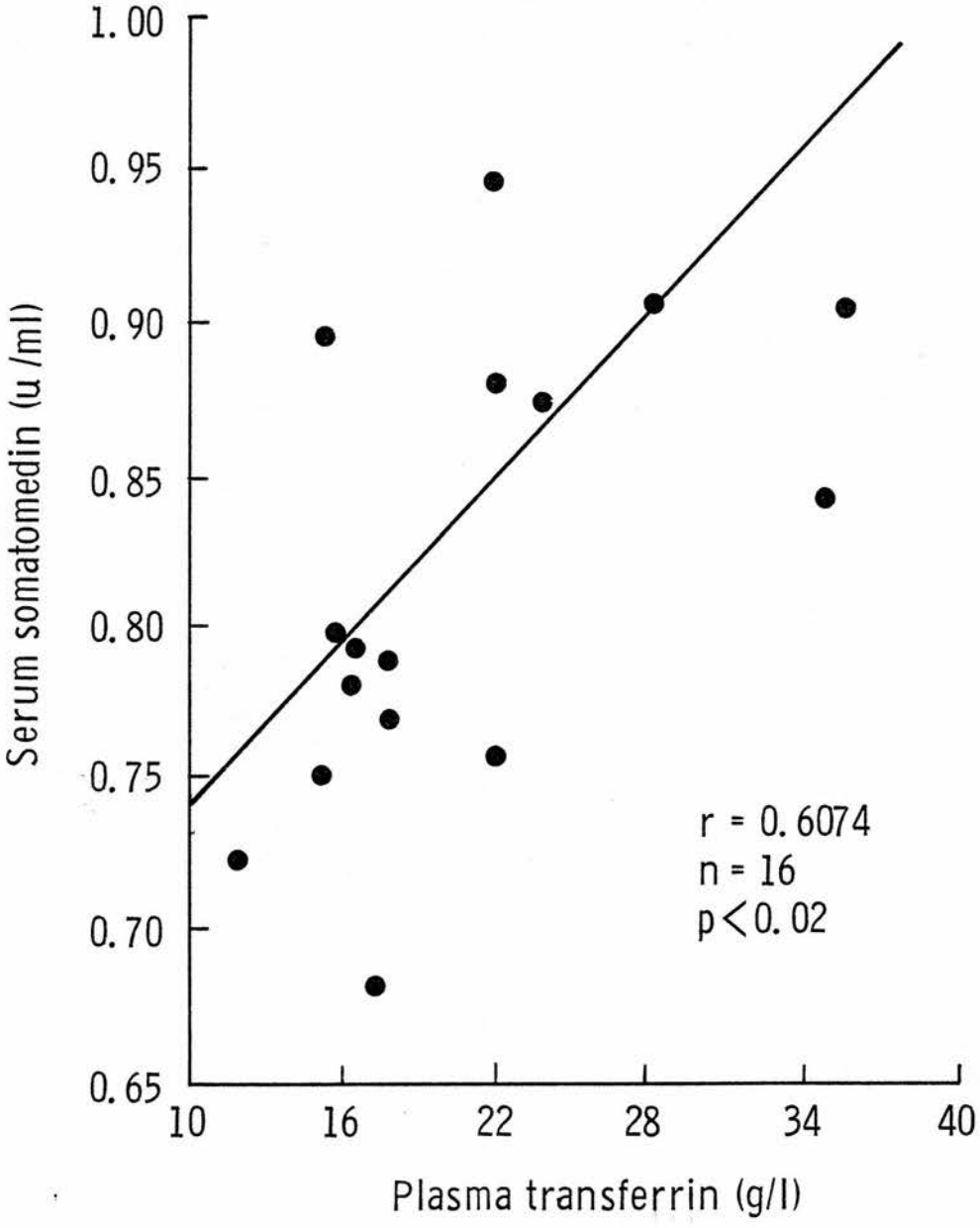

Fig. 4. Relationship between plasma transferrin concentration and serum somatomedin activity.

TABLE 13

Patient No.	Serum Somatomedin (u/ml)
1	0.78
2	0.85
3	0.68
4	0.89
5	0.77
6	0.79
7	0.76
8	0.79
9	0.72
10	0.80
11	0.75
12	0.91
13	0.86
14	0.85
15	0.93
16	0.91
mean	0.81
S.D.	0.07

TABLE 13: Serum somatomedin activity in the patients.

Thyroid Function Tests

All the patients were clinically euthyroid by physical examination and none had either exophthalmos or a goitre at the time of study.

Table 14 shows the mean values ($\pm$ 1 S.D.) of plasma thyroxine (T_4), triiodothyronine (T_3), thyrotrophin (TSH), T_3 resin uptake (T_3RU) and free thyroxine index (FTI) levels as determined at the start, middle and end of period of study. The differences in the three measurements were not statistically significant (paired t-test). Individual patients data are presented in Table 3 of Appendix (A).

TABLE 14

	T_4	T_3	T_3RU	FTI	TSH*
	(nmol/l)	(nmol/l)	(%)		(mu/l)
Normal range	70-190	1.2-3	92-117	70-180	0-5
S	104.7 ± 18.0	2.24 ± 0.62	107.4 ± 7.9	98.2 ± 19.3	2.16 ± 0.8
M	100.1 ± 16.6	2.12 ± 0.51	107.7 ± 7.8	91.1 ± 15.7	2.4 ± 1.4
E	100.7 ± 18.6	2.42 ± 0.70	105 ± 13.4	95.2 ± 24.8	2.2 ± 0.71

TABLE 14: Mean (± 1 S.D.) plasma thyroid hormone levels in the patients determined at start (S), middle (M) and end (E) of period of study.

* excluding the two cystinotic patients.

The range and mean plasma values for T_4, T_3, TSH, T_3RU and FTI in patients, measured in mid-period sampling, and in controls are shown in Table 15.

All patients had plasma T_4 concentrations within normal range for the assay; however, five patients (numbers 1, 3, 10, 15, 16) were below the mean-1 S.D. of the control group and the mean plasma T_4 in the patients as a group was significantly lower than in the normal controls (Table 15). None of the five patients with plasma T_4 concentrations at the lower end of normal had abnormal FTI.

Although in the majority of patients T_3 levels were within the normal range for the assay, with the exception of patient number 11 whose T_3 was below the lower limit of normal, seven (numbers 1, 2, 5, 8, 9, 11, 12) had levels below the mean - 1 S.D. for the controls and the mean plasma T_3 concentrations were significantly lower in the patients than in the controls (Table 15). Thirteen of the sixteen patients had TSH levels below the upper limit of normal for the assay. One patient (number 7) had slightly increased TSH and two patients (numbers 13, 14), who were both cystinotic, had a considerably elevated plasma TSH concentration. Excluding these two patients the mean plasma TSH concentration was similar in patients and controls (Table 15). The two patients with cystinosis and elevated plasma TSH had normal plasma values for T_4 and T_3. None of the patients who had low normal T_4 or T_3 had elevated plasma TSH.

Plasma T_3RU and FTI were within normal range. In comparison to controls, mean T_3RU was not statistically different but mean FTI was significantly reduced (Table 15).

TABLE 15

Test	Patients		Controls		t	p*
	Results (mean ± SD)	Range	Results (mean ± SD)	Range		
T_4 (nmol/l)	100.1 ± 16.6	70.8-119.7	114.6 ± 18.8	86-150	-2.38	< 0.05
T_3 (nmol/l)	2.12 ± 0.51	1.1-2.86	2.55 ± 0.45	1.95-3.74	-2.504	< 0.02
T_3RU (%)	107.7 ± 7.8	93 - 121.3	106.6 ± 6.7	95.8-115	+1.046	NS
FTI	91.1 ± 15.7	70.6-118.4	107.3 ± 16.3	85.3 - 130.4	-2.94	< 0.01
TSH** (mu/l)	2.4 ± 1.4	0.9-6.3	2.75 ± 1.2	1.5 - 5.9	-0.74	NS

TABLE 15: Thyroid function tests in patients (mid-period sampling) compared to controls. Values are mean ± 1 SD. The absolute range is also included.

* The significance of the difference between patients and controls was tested by student t-test.

**Excluding the two cystinotic children.

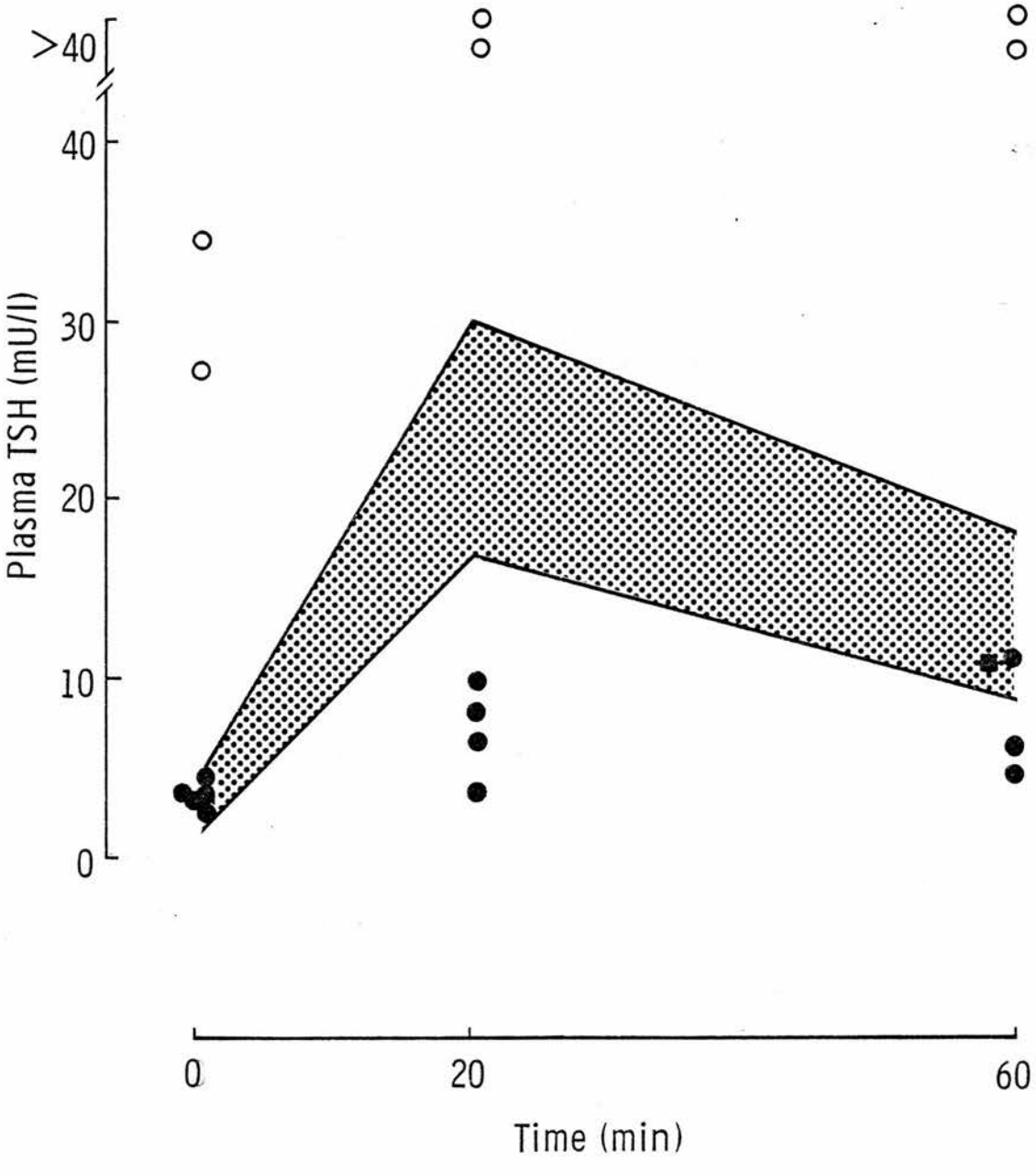

FIG. 5 TSH response to TRH stimulation in 6 of the patients. Open circles represent the two boys with cystinosis. Shaded area indicates the normal range.

Analysis of thyroid hormone in relation to the other metabolic hormones, duration and amount of dialysis, plasma urea and creatinine concentrations and nutritional status of the patients as indicated by food intake, plasma albumin, transferrin and C3 concentrations revealed no significant relationships.

TRH - Stimulation Test

After TRH injection, plasma TSH concentration rose rapidly in the seven normal children reaching a peak by 20 minutes. Of the six patients tested, the two patients who had cystinosis (numbers 13, 14) showed an excessive TSH response and the other four patients had blunted increases in plasma TSH with delayed peak in two of them, (Figure 5). The basal TSH level was markedly elevated in the two cystinotic patients and within normal range for both the control group and the non-cystinotic patients.

SEX HORMONES

LH, FSH and testosterone

At the time of sampling, the patients were assigned to puberty stages (P 1 = prepubertal; P 1-5 = pubertal) as described in methods. Estimations of plasma LH, FSH and testosterone levels determined at the start and end of the period of study were pooled according to sex and puberty status and compared to a range of reference values obtained from normal children of the same sex and stage of sexual development (Figure 6). Plasma LH was elevated in both prepubertal and pubertal boys, while plasma FSH was within normal range in the prepubertal boys and only three of the eight estimates in pubertal boys showed elevated levels.

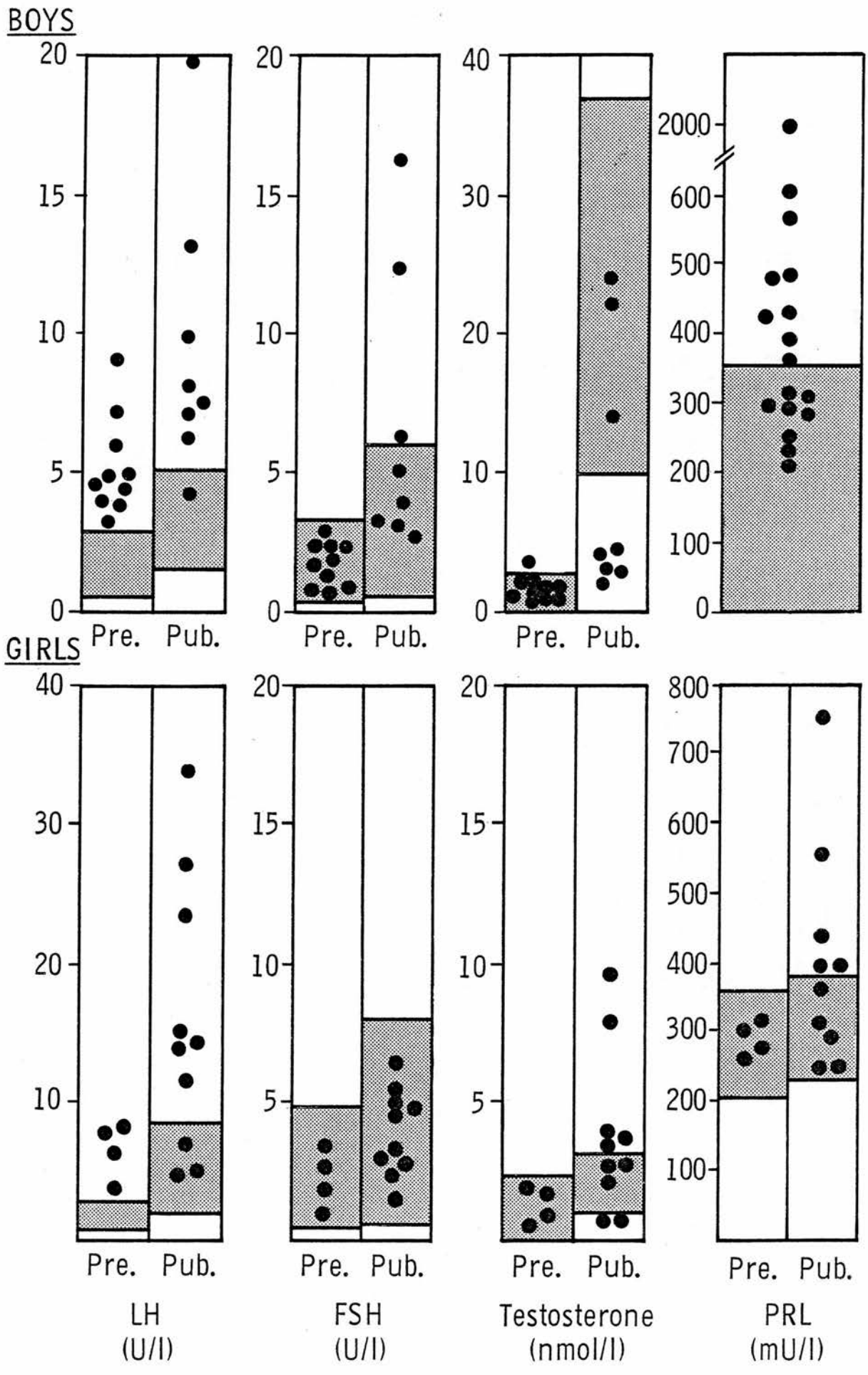

FIG. 6 Plasma sex hormone levels in Prepubertal (Pre.) and Pubertal (Pub.) boys and girls determined at the start and end of period of growth observation. Stippled areas represent the normal ranges. PRL = Prolactin.

Plasma testosterone levels were normal in the prepubertal boys and decreased in five of the eight estimates (60.2%) in the pubertal boys. In the girls, plasma LH was elevated in the prepubertal and in most of the pubertal girls, while normal plasma FSH levels were found in both groups; plasma testosterone concentration was within normal range in the prepubertal and variable in the pubertal girls, with increases in five and subnormal levels in two of the ten estimates.

There was a significant positive correlation between plasma LH and testosterone levels in the boys ($r = 0.794$, $p < 0.001$) but not in the girls ($r = 0.161$, $p > 0.05$). Similarly, plasma FSH correlated significantly with plasma testosterone in the boys ($r = 0.886$, $p < 0.001$) and not in the girls ($r = 0.365$, $p > 0.05$). In the girls, mean plasma LH and FSH levels over the period of study correlated positively with mid-period chronological age ($r = 0.706$, $p < 0.05$; $r = 0.632$, $p > 0.05$ respectively). Higher correlation coefficients were obtained when plasma LH and FSH levels were related to mid-period bone age ($r = 0.809$, and $r = 0.895$ respectively). Although plasma gonadotrophin tended to increase with age in the boys, the relationship was not statistically significant ($r = 0.38$, $p > 0.05$). Correlations between sex hormone levels and growth are presented below in the section entitled "Hormones and growth".

PROLACTIN

Thorner et al. (1977) have shown that in normal boys there was no significant difference in plasma prolactin levels at any stage of pubertal development. Thus, plasma prolactin estimates were grouped according to puberty status in the girls but not in the boys. These

are shown in Figure 6. Raised levels were found in nine of the seventeen estimates in the boys (53%) and in three of ten estimates in the pubertal girls. In the prepubertal girls, plasma prolactin levels were normal. The elevated levels were not manifested by galactorrhoea in any of the patients.

LH/FSH - RH Stimulation Test

The individual gonadotrophin responses to the intravenous injection of 100 ug of synthetic luteinizing hormone releasing hormone in four prepubertal or early pubertal boys (Stage 1 - 2) are shown in Table 16 and compared to mean responses in six normal boys of comparable age and sexual development. The results in a male patient in advanced puberty (Stage 4) are also shown. The mean basal LH level was significantly elevated in the patients compared to controls ($p < 0.001$). Mean plasma LH level at 20 minutes following LH-RH injection was similar in patients and controls. However, the mean rise above basal level was greater in the control group than in the patients (8.1 ± 7.2 u/l and 4.4 ± 2.64 u/l respectively) but the difference was not statistically significant. Mean LH level and increment at 60 minutes were significantly higher in the patients compared to controls ($p < 0.02$; $p < 0.05$ respectively). The patient in advanced puberty (number 10) showed augmented LH responses at 20 and 60 minutes. In general, LH responses to LH-RH were normal but delayed in the boys in pre- and early puberty and excessive in the pubertal boy.

Mean basal plasma FSH level was significantly higher in the patients compared to controls ($p < 0.001$) but the mean response to LH-RH stimulation was blunted and delayed in comparison to the control

TABLE 16

	PLASMA LH (u/l)					PLASMA FSH (u/l)				
	Time (min.)									
PATIENTS No.	0	20	Δ 20	60	Δ60	0	20	Δ 20	60	Δ60
PUBERTAL STAGE 1-2										
5	5.7	11.7	6	25.7	20.7	1.4	2	0.6	1.9	0.5
7	4.6	5.1	0.5	7.8	3.2	1.3	1.2	-0.1	1.8	0.5
13	4.3	9.5	5.2	20.4	16.1	0.8	0.9	0.1	2.4	1.6
14	5	10.9	5.9	30.1	25.1	1.5	1.4	-0.1	2.4	0.9
MEAN	4.9	9.3	4.4	21	16.3	1.25	1.37	0.13	2.13	0.87
S.D.	0.6	2.9	2.6	9.6	9.4	0.31	0.46	0.33	0.32	0.52
CONTROLS(No.6)										
MEAN	0.7	8.8	8.1	6.3	5.6	0.25	3.7	3.45	2.75	2.5
S.D.	0.4	7.3	7.2	5.7	5.5	0.14	1.46	1.48	1.28	1.3
SIGNIFICANCE (P)	<0.001	NS	<0.1	<0.02	<0.05	<0.001	<0.01	<0.002	NS	<0.05
PUBERTY STAGE 4										
10	8.7	40	31.3	59	50.3	6.2	9.9	3.7	13.2	7.0

TABLE 16: GONADOTROPHIN RESPONSE TO iv. LH-RH (100 ug) IN 5 MALE PATIENTS AND IN 6 NORMAL BOYS.

Δ = Increment SD = Standard Deviation

TABLE 17

		PATIENTS		CONTROLS		t	p
		MEAN	S.D.	MEAN	S.D.		
TG (mmol/l)	S	1.867	0.67	0.838	0.25	+5.92	<0.001
	M	2.018	0.87			+5.51	<0.001
	E	1.721	0.72			+4.83	<0.001
CHOL (mmol/l)	S	6.32	0.75	4.49	0.82	+6.27	<0.001
	M	6.28	1.27			+4.71	<0.001
	E	6.01	0.98			+4.45	<0.001
NEFA (ueq/l)	S	560.8	86	814.4	343	-2.59	<0.02
	M	705.7	228			-1.08	NS
	E	575.6	103			-2.32	<0.05
GLYCEROL (mg/dl)	S	1.01	0.33	1.21	0.28	-1.75	<0.1
	M	0.94	0.29			-2.67	<0.02
	E	0.95	0.20			-2.68	<0.02
BG (mmol/l)	S	5.02	0.58	4.28	0.54	+3.46	<0.002
	M	4.96	0.40			+3.96	<0.001
	E	5.11	0.53			+4.05	<0.001

TABLE 17: Mean ($\pm$ 1 S.D.) plasma triglycerides (TG), cholesterol (CHOL), non-esterified fatty acids (NEFA), serum glycerol and blood glucose (BG) concentrations in patients and controls.
S = start; M = middle; E = end of period of study.

group. Patient number 10 had elevated basal FSH level but the response was normal, though delayed for his puberty status.

BLOOD GLUCOSE (BG)

Mean $\pm$ S.D. fasting blood glucose concentrations in the patients measured at the start, middle and end of study are shown in Table 17. Values in individual patients are presented in Table 4 of Appendix (A). In comparison to control subjects, the three sets of basal blood glucose levels in patients were significantly increased (Table 17). Paired analysis of the data (Table 18) showed no significant difference between the three sets of mean values. The middle of the period values were, therefore, used, as appropriate, for further analysis.

TABLE 18

	S vs M		S vs E		M vs E	
	t	p	t	p	t	p
TG	-1.5475	NS	0.8709	NS	3.4177	<0.01
CHOL	-0.5679	NS	0.8537	NS	2.5961	<0.05
NEFA	-2.1877	<0.07	-0.1734	NS	2.3276	<0.05
GLYCEROL	0.5831	NS	-0.1803	NS	1.1042	NS
BG	0.3223	NS	-0.948	NS	-1.27	NS

TABLE 18: Significance of variation in plasma triglycerides (TG), cholesterol (CHOL), non-esterified fatty acids (NEFA) and serum glycerol and blood glucose (BG) concentrations determined at start (S), middle (M) and end (E) of period of study (paired t-test).

Blood Glucose and metabolic hormones

Table 19 shows the correlation coefficients between fasting blood glucose and the simultaneously measured plasma insulin, growth hormone and cortisol levels. There was a positive correlation between blood glucose and plasma cortisol significant at 5% level and although there was a tendency for patients with high blood glucose to have raised plasma insulin levels, this linear relationship was not significant. Blood glucose concentration did not relate to plasma growth hormone level.

TABLE 19

	Insulin		Growth hormone		Cortisol	
	v	p	v	p	v	p
TG	+0.57	< 0.02	-0.138	NS	+0.161	NS
CHOL	+0.038	NS	-0.324	NS	-0.397	NS
NEFA	-0.198	NS	+0.610	< 0.02	-0.231	NS
GLYCEROL	-0.212	NS	+0.275	NS	-0.288	NS
BG	+0.306	NS	-0.024	NS	+0.556	<0.05

TABLE 19: Relationships between plasma insulin, growth hormone and cortisol levels and plasma lipid and blood glucose concentrations (mid-period samples).
TG = triglycerides; CHOL = cholesterol;
NEFA = non-esterified fatty acids;
BG = blood glucose.

Blood glucose and haemodialysis

Relationships between basal blood glucose and the duration and amount of dialysis, plasma urea and creatinine concentrations were tested. No significant correlation between blood glucose and these various parameters was found.

Blood glucose and diet

Neither the intake of total energy, protein, carbohydrate and fat (recorded nearest to sampling) at the middle of the period nor the average intake over the year had any demonstrable influence on blood glucose levels.

PLASMA LIPIDS

Table 17 shows patients mean $\pm$ S.D. plasma triglycerides (TG), cholesterol (CHOL) and non-esterified fatty acids (NEFA) concentrations and serum glycerol levels measured at the start, middle and end of the period of observation and compared to mean values of the control group. Individual patient's values are presented in Table 5 of Appendix (A).

There were consistent increases in both plasma TG and cholesterol concentrations in patients compared with controls. The differences were statistically significant (Table 17). No relationship was found between plasma lipid levels and age or sex of the patients or their body mass index (weight/height ^{2}, Keys et al., 1972), or the duration or amount of dialysis. No patient had detectable chylomicrons or a broad beta-band on lipoprotein electrophoresis. Type IV hyperlipoproteinaemia was the predominant finding occurring in ten of the children. Four had type II b and two had normal patterns, (Table 5 of Appendix (A)). The plasma NEFA levels were significantly decreased at the beginning and end

of the study and although lower than controls in samples obtained at middle of the period, the difference was not significant. However, the mean of the 3 measurements was significantly lower than controls ($p < 0.05$). In the 2nd and 3rd samplings, serum glycerol concentrations were significantly decreased in the patients compared with controls (Table 17), and the mean of the 3 estimates was significantly lower in the patients ($t = 3.05$, $p < 0.005$).

Plasma Lipid Changes

TG

When tested by 't' test for paired variation (Table 18), the mid-period TG concentrations were found to be significantly higher than end period values ($t = 3.41$; $p < 0.01$) and although increased in comparison to the values obtained at the start of study, the difference was not statistically significant.

Cholesterol

The mean plasma cholesterol concentration at the end of the period of study was significantly lower than mid-period value, but similar to that obtained at the start of the study (Table 18).

NEFA

Plasma NEFA levels increased in mid-period in comparison to the initial values, but the difference was only of border line significance ($t = 2.1877$, $p = < 0.07$). There was, however, a significant decrease in the end period compared with mid-period levels ($t = -2.3276$, $p < 0.05$) (Table 18).

Serum Glycerol

No significant changes were seen in serum glycerol concentrations.

Plasma Lipids - metabolic hormones interrelationships

Table 19 shows the interrelationships between plasma lipid levels and the simultaneously measured plasma insulin, growth hormone and cortisol concentrations. There was a linear relationship between plasma insulin levels and TG concentrations. This correlation was significant ($r = 0.57$; $p < 0.02$) in mid-period samples (Figure 7), and was further demonstrated when the mean plasma insulin concentration over the year of study was related to the mean plasma TG over the same period ($r = 0.4917$; $p < 0.05$). The close time-relationship between changes in the concentration of plasma insulin and TG is shown in Figure 8. It is apparent that the relative changes in plasma TG and insulin concentration are closely related. There was no significant relationship between plasma TG and the other metabolic hormones. While plasma cholesterol and serum glycerol concentrations did not relate to any of the metabolic hormones, plasma non-esterified fatty acids bore an inverse relationship to plasma insulin concentrations at the start and the end of period samplings which was of border line significance ($r = -0.504$, $p < 0.1$; $r = -0.413$, $p < 0.1$ respectively) and positively correlated with plasma growth hormone at the middle of the period ($r = +0.610$, $p < 0.01$) (Figure 9).

Effect of diet

The relationship between plasma TG and cholesterol concentrations, determined at the middle of the period, and dietary intakes of total energy, carbohydrates, total fat and proteins, recorded closest to the

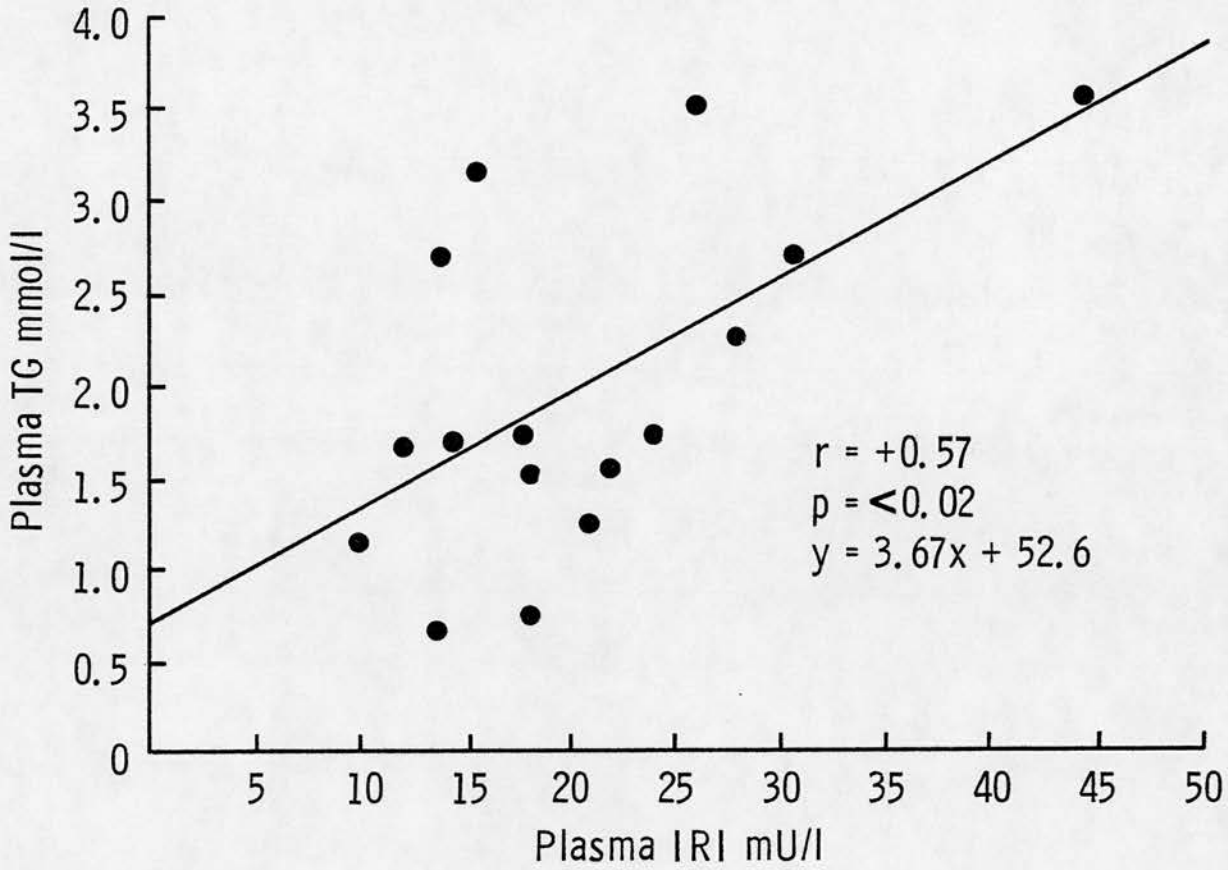

Fig. 7. Relationship between plasma triglyceride (TG) concentration and plasma insulin (IRI) levels.

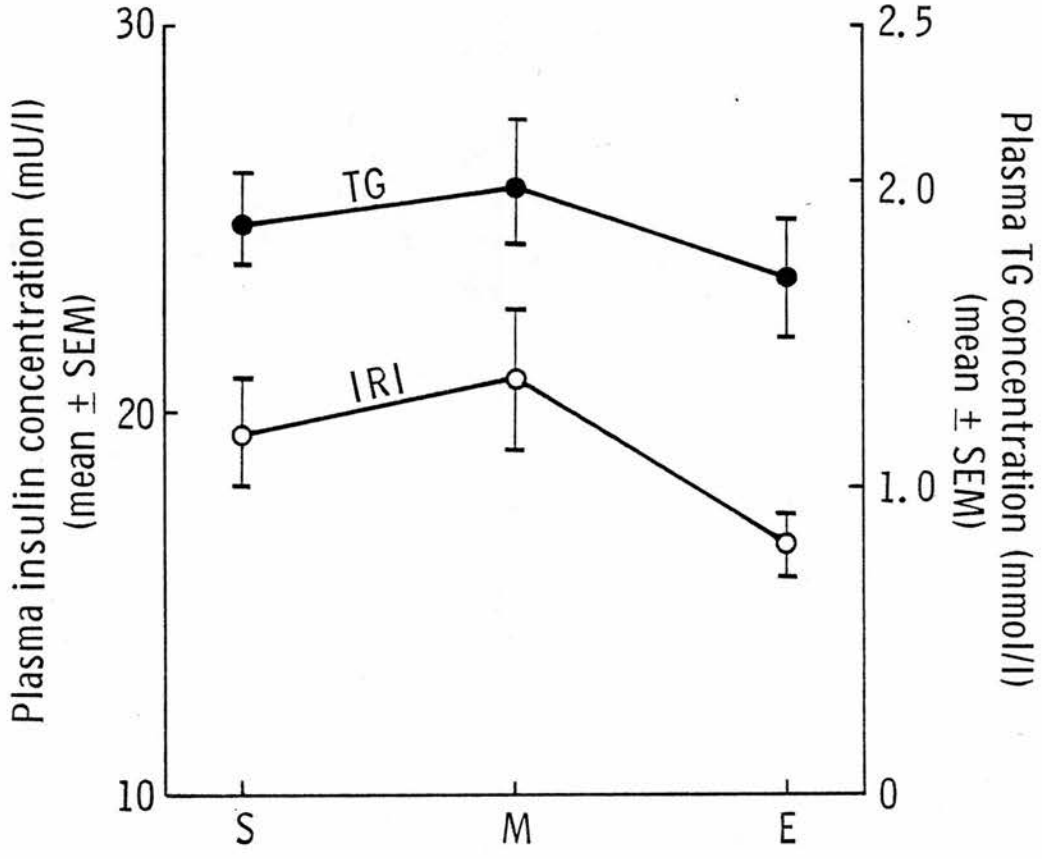

FIG. 8 Relative changes in plasma triglyceride (TG) and insulin (IRI) concentrations. S = start; M = middle; E = end of the period of study.

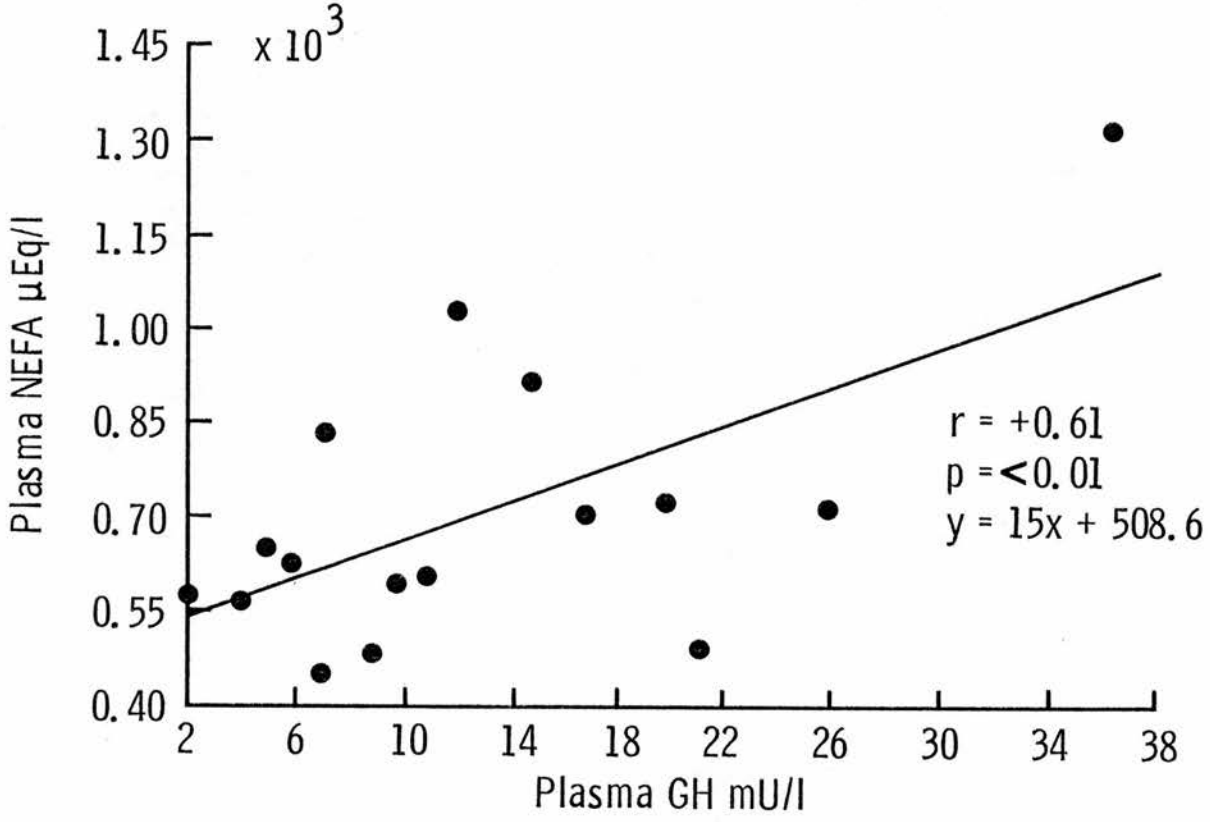

Fig. 9. Relationship between plasma growth hormone (GH) levels and plasma nonesterified fatty acids (NEFA) concentrations.

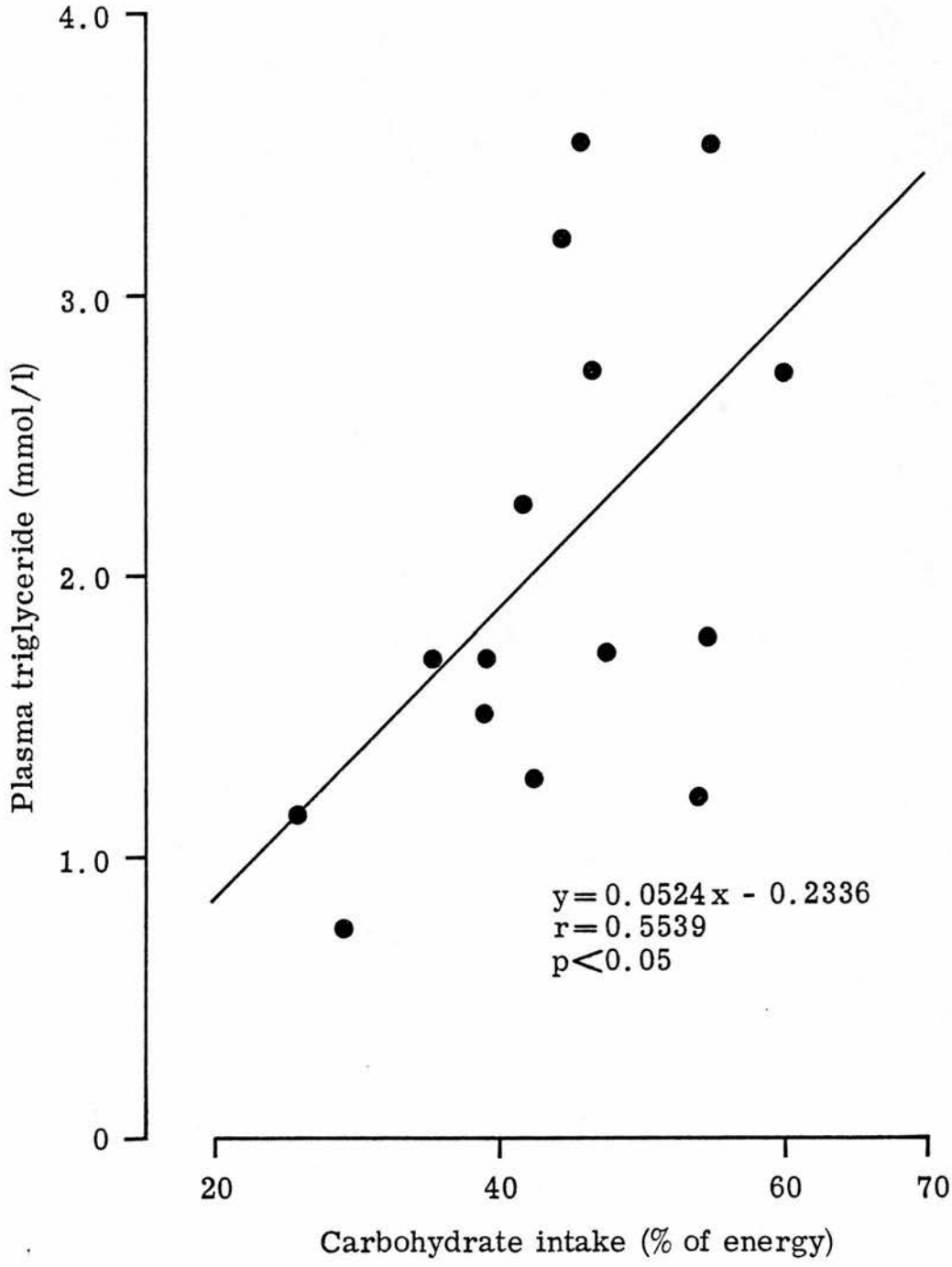

Fig. 10. Relationship between plasma triglyceride concentration and carbohydrate intake (% of total energy intake).

time of samplings, is shown in Table 20. There was a tendency for those children with lower intakes of total energy, fats and protein to have higher TG levels. There was significant positive correlation between plasma TG and the percentage of calories derived from carbohydrates ($r = 0.55$, $p < 0.05$) (Figure 10). No correlation between plasma cholesterol concentration and food intakes could be shown. A strikingly similar relationship between food intake and plasma lipids were obtained when the mean intake over the year of study was related to the mean plasma TG and cholesterol concentrations over the same period (Table 21).

PLASMA AMINO ACIDS

The mean ($\pm$ 1 S.D.) of the fasting plasma amino acid concentrations in the patients determined at the three time points and compared with control values are shown in Table 22.

There were consistently significant reductions in concentrations of valine, leucine, isoleucine, lysine, tyrosine and histidine. Glycine and alanine were significantly elevated, while the remainder were within normal limits. The ratio of tyrosine to phenylalanine was consistently reduced, mainly due to the reduction in tyrosine concentration. The valine/glycine ratio was also low because of the reduced valine concentration and raised glycine concentration.

Plasma amino acid changes

Comparison by paired analysis of plasma amino acid concentrations at the start, middle and end of period of observation (Table 23) shows significant increase in mean plasma valine concentration at the end of

TABLE 20

	Total Energy		Fat		Carbohydrate		Protein	
	r	p	r	p	r	p	r	p
Triglycerides	-0.37	NS	-0.46	<0.1	-0.12	NS	-0.38	NS
Cholesterol	-0.13	NS	-0.06	NS	-0.18	NS	+0.13	NS

TABLE 20: Relationship between diet (as % R.I. for height-age) and plasma lipid concentrations.

TABLE 21

	Total energy (% R.I.)		Fat (% R.I.)		Carbohydrate (% R.I.)		Carbohydrate (% of energy)		Protein (% R.I.)	
	r	p	r	p	r	p	r	p	r	p
Triglycerides	-0.127	NS	-0.185	NS	-0.100	NS	+0.531	<0.05	-0.302	NS
Cholesterol	+0.064	NS	+0.023	NS	+0.002	NS	-0.092	NS	-0.005	NS

TABLE 21: Relationship between mean dietary intake during the year of study and mean plasma lipid concentrations over the same period.
R.I. = recommended intake.

TABLE 22

AMINO ACID (μmol/l)		PATIENTS $\bar{x}$	S.D.	No	CONTROLS $\bar{x}$	S.D.	No	t	p
VALINE	S	142.7	28.6	(9)				4.17	<0.001
	M	147.6	31.7	(16)	197	32	(31)	5.62	<0.001
	E	170.8	35.3	(15)				2.43	<0.02
LEUCINE	S	79.2	17.6	(9)				4.56	<0.001
	M	68.6	12.5	(16)	100	16	(31)	7.15	<0.001
	E	77.8	17.1	(15)				4.22	<0.001
ISOLEUCINE	S	40.3	10.3	(9)				3.86	<0.001
	M	41.9	12.8	(16)	55	10	(31)	3.50	<0.002
	E	44.2	9.8	(15)				3.48	<0.002
METHIONINE	S	13.2	4.9	(9)				2.53	<0.05
	M	13.6	4.0	(14)	18	7	(25)	2.51	<0.02
	E	10.75	3.8	(11)				2.21	<0.05
PHENYL-ALANINE	S	38.6	9.7	(9)				0.97	NS
	M	38.5	9.1	(16)	42	7	(28)	1.14	NS
	E	40	8.5	(15)				0.31	NS
LYSINE	S	175	26.8	(9)				1.7	NS
	M	154.6	23	(15)	156	31	(28)	0.11	NS
	E	164	35.7	(13)				0.69	NS
HISTIDINE	S	79.3	16	(9)				1.14	NS
	M	70.3	10.6	(16)	86	13	(28)	4.33	<0.001
	E	72.6	11.9	(14)				3.34	<0.002
GLYCINE	S	456	90	(9)				6.61	<0.001
	M	405	113	(16)	251	44	(31)	5.24	<0.001
	E	403	117	(15)				4.87	<0.001
ALANINE	S	447	158.5	(9)				3.16	<0.005
	M	380	149.4	(16)	276	69	(30)	2.62	<0.02
	E	380.2	169.3	(15)				2.29	<0.05
TYROSINE	S	20.3	6.9	(9)				9.68	<0.001
	M	23	5.8	(16)	43	9	(28)	11.06	<0.001
	E	20.9	4.7	(15)				12.9	<0.001

TABLE 22: Plasma amino acid concentrations in patients at start (S), middle (M) and end (E) of period of study and in controls.

TABLE 23

	VALINE	LEUCINE	ISOLEUCINE	METHIONINE	PHENYL-ALANINE	LYSINE	HISTIDINE	GLYCINE	ALANINE	TYROSINE
S vs M	t	0.4208	0.2809	-0.5430	-0.3653	0.9954	2.1688	0.9196	1.9634	1.5119	-1.2697
	p	NS	NS	NS	NS	NS	< 0.1	NS	< 0.1	<0.2	NS
M vs E	t	-3.3643	-1.653	-0.3917	2.4229	-0.7605	-1.0088	-0.6887	-0.2628	-0.5049	+1.3096
	p	P 0.005	< 0.1	NS	< 0.05	NS	NS	NS	NS	NS	< 0.2
S vs E	t	-0.9081	-1.2127	-1.9090	1.9283	-0.1529	0.6674	1.1648	1.4229	0.7492	-0.0922
	p	NS	< 0.5	< 0.1	< 0.1	NS	NS	NS	< 0.2	NS	NS

TABLE 23: Significance of variation in plasma amino acid concentration estimated at start (S), middle (M) and end (E) of period of study. Paird t-test.

the period compared with the middle of the period ($t = 3.364$, $p < 0.005$), although the levels remained significantly lower than normal. The other branched chain amino acids, leucine and isoleucine showed a similar trend but the changes in their concentrations did not reach statistical significance. There was also significant reduction in plasma methionine concentration at the end of period compared with mid-period values ($t = 2.422$, $p < 0.05$), No significant changes in other amino acids were noted although both plasma glycine and alanine concentrations tended to decrease at the middle and end of the period in comparison with their intiial levels.

Figure 11 shows the relative changes in plasma branched chain amino acids, valine, leucine and isoleucine and the non-essential amino acids, alanine and glycine during the period of study. The decline in plasma level of both alanine and glycine was followed by a reciprocal increase in valine, leucine and isoleucine concentrations. As all patients were sampled at the middle of the period and since there were no statistically significant changes in amino acid concentrations during the period of study with the exception of valine and methionine, the values obtained at this time were used for further analysis.

Amino acids and residual renal mass and degree of uraemia

Two patients were surgically anephric (numbers 7, 9) and analysis of their results showed no difference when compared with the remainder. No plasma amino acid concentration correlated with the duration or the amount of dialysis. There was no correlation between plasma creatinine and any of the amino acids but plasma urea concentrations were correlated

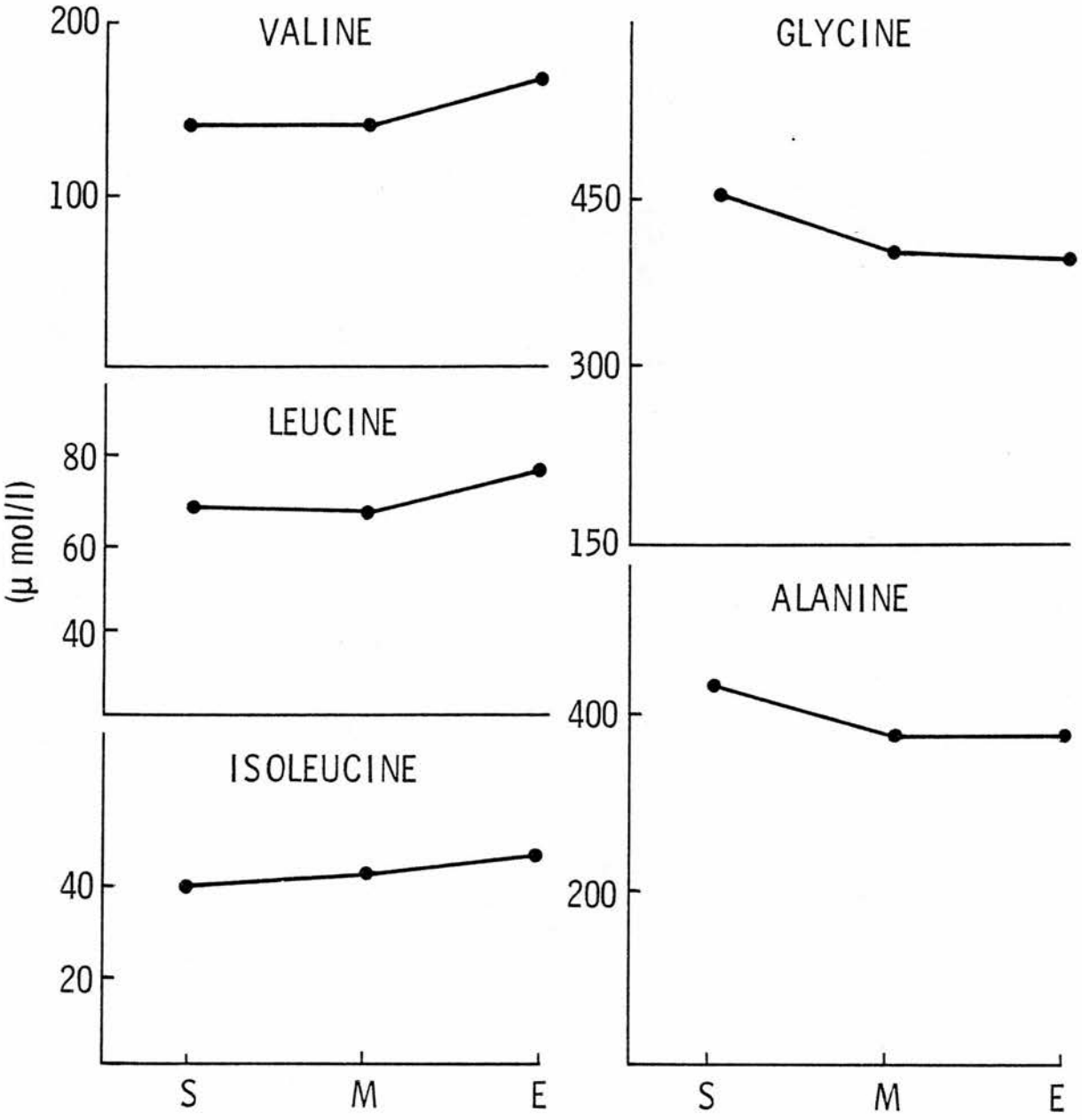

FIG. 11 Relative changes in plasma branched-chain amino acids and alanine and glycine levels during the period of study. S = start; M = middle; E = end of the period.

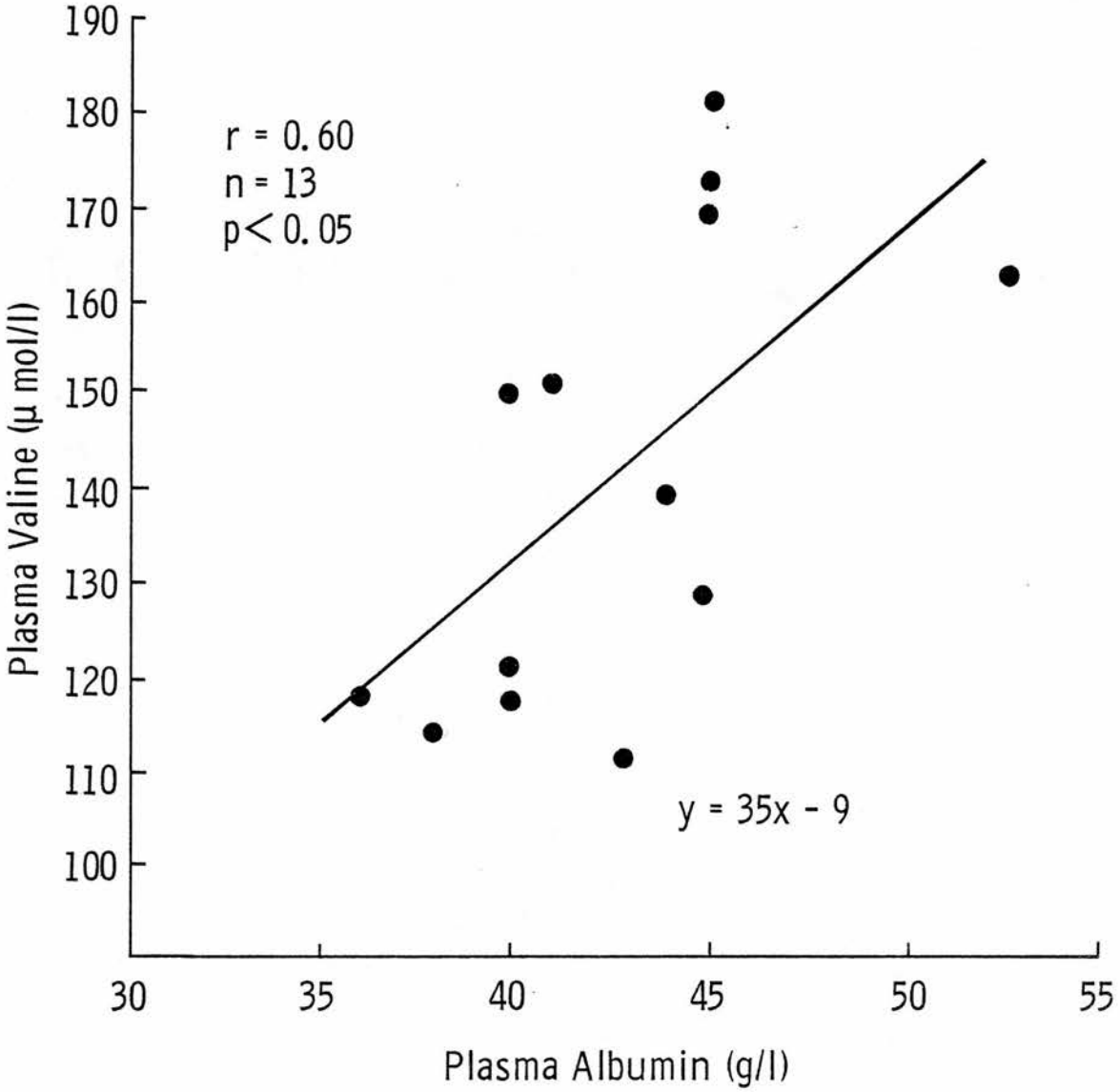

FIG. 12 Relationship between plasma albumin and plasma valine.

inversely, both with the plasma glycine ($r = -0.6031$; $p < 0.05$) and plasma alanine concentrations ($r = -0.6060$; $p < 0.05$).

Effect of diet and nutritional state

Table 24 shows the relationship between some plasma amino acids and the dietary intakes assessed closest to the time of mid-period sampling. There was an inverse linear relationship between glycine and total energy, fat and protein. The valine/glycine ratio showed a similar, though not as close a relationship, and the plasma valine was not influenced by the diet at all. However, there was a direct linear correlation between plasma valine concentration and plasma albumin, significant at the 5% level (Figure 12). Other amino acids did not correlate with plasma albumin and there was no relationship between amino acid concentrations and plasma transferrin and C3 concentrations.

Amino acids and the metabolic hormones

Figure 13 illustrates the relative changes in mean plasma insulin, GH and cortisol levels and mean plasma valine, leucine and the gluconeogenic amino acid alanine during the year of study.

At the start of study, plasma insulin concentration was inversely related to both plasma valine and leucine concentrations, ($r = -0.42$ and $r = -0.50$ respectively). However, these relationships did not reach conventional level of significance ($p < 0.1$). Similarly the decline in plasma insulin concentration towards the end of the period of study was associated with an increase in plasma valine and a decrease in plasma alanine levels, but this was not statistically significant and there was no significant correlation between insulin and any of the other amino acids.

TABLE 24

Amino acid	Energy		Carbohydrate		Fat		Protein	
	r	p	r	p	r	p	r	p
Glycine	-0.55	<0.05	-0.25	NS	-0.60	<0.05	-0.41	NS
Valine	+0.22	NS	+0.14	NS	+0.14	NS	+0.19	NS
Valine/ Glycine	+0.55	<0.05	+0.19	NS	+0.56	<0.05	+0.34	NS

TABLE 24: Relationship between diet, as percentage of recommended intake for height age, and plasma amino acid concentrations.

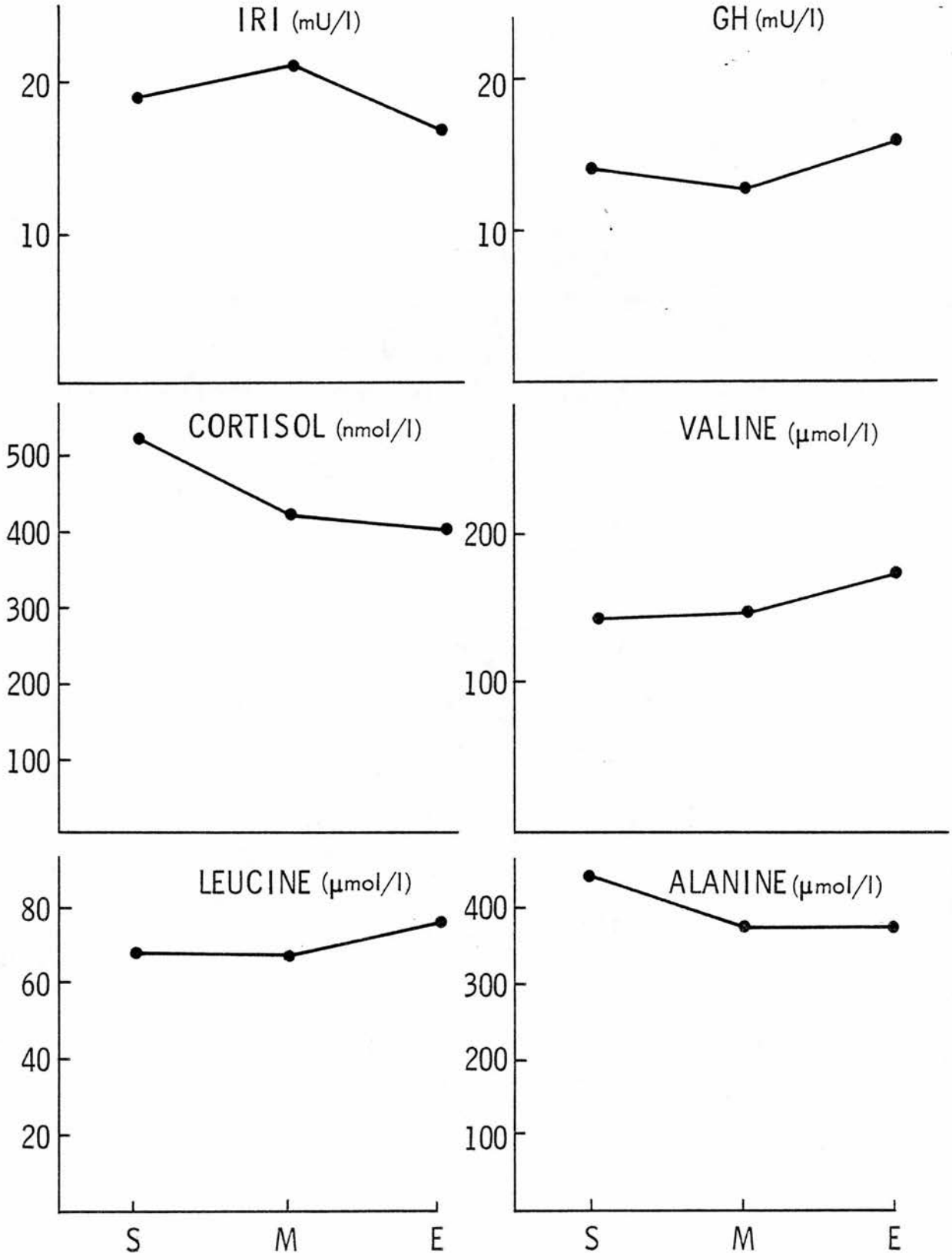

FIG. 13 Relative changes in mean plasma plasma insulin (IRI), growth hormone (GH), cortisol levels and the plasma amino acids valine, leucine and alanine during the period of study. S = start; M = middle; E = end of the period.

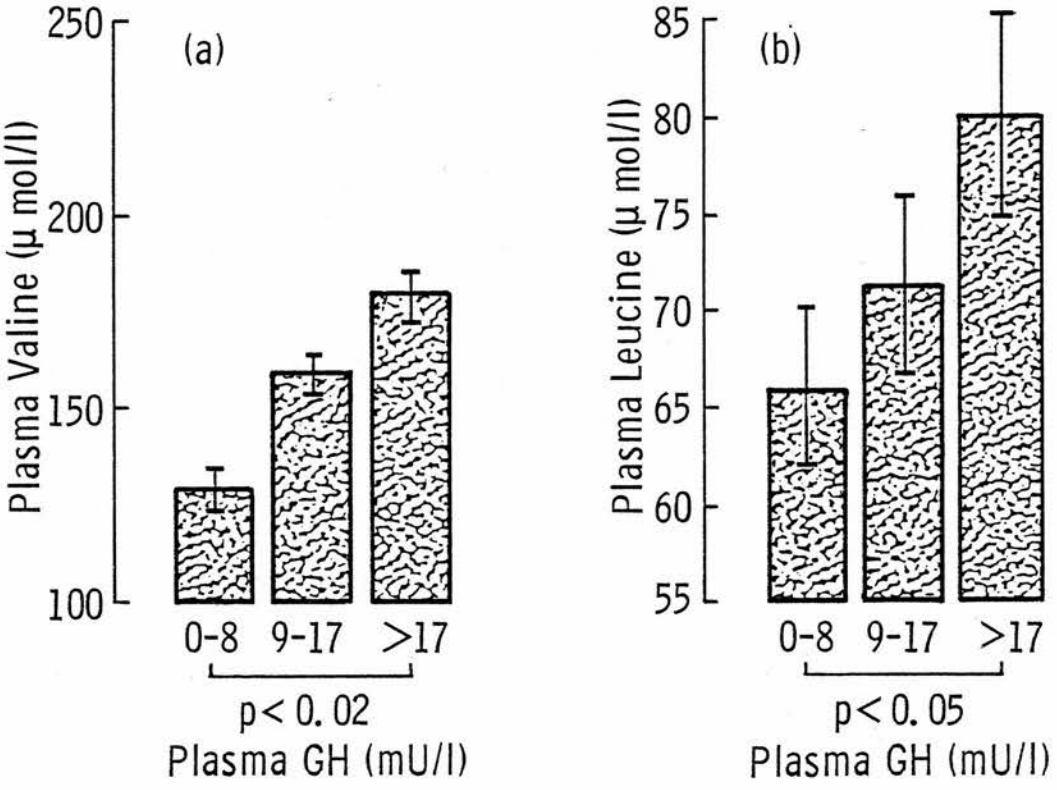

FIG. 14 Relationship between plasma growth hormone (GH) and (a) Plasma Valine and (b) Plasma Leucine concentrations. Values are the means with their standard errors.

Mean plasma GH levels tended to rise as plasma valine began to increase and plasma alanine to decrease. These relationships were not statistically significant. However, when analysed cross-sectionally by grouping the mean plasma valine values according to mean plasma GH concentrations, patients with higher GH levels were found to have significantly higher valine concentrations ($t = 2.919$; $p < 0.02$) (Figure 14). A similar relationship was found between GH and plasma leucine (Figure 14), but no other amino acids correlated significantly with GH.

The direction of change in plasma branched chain amino acids was opposite to that of plasma cortisol whereas plasma alanine changes were in the same direction as those of plasma cortisol. Regression analysis of plasma cortisol and the amino acids at the three time points revealed no significant correlation.

GROWTH AND DEVELOPMENT

Figures 15 and 16 show the observed heights of the boys and girls respectively for the beginning and end of period of observation. They have been plotted against (a) chronological age and (b) bone age and are numbered as in Table 1.

The mid-period chronological and bone ages, the height, the height standard deviation score (HSDS) for mid-period chronological and bone ages and the puberty ratings at the beginning and end of the period are given in Table 25. The observed height velocity (cm/year), the growth velocity standard deviation score (GVSDS) for mid-period bone age and growth rating, as described in the method, are detailed in Table 26.

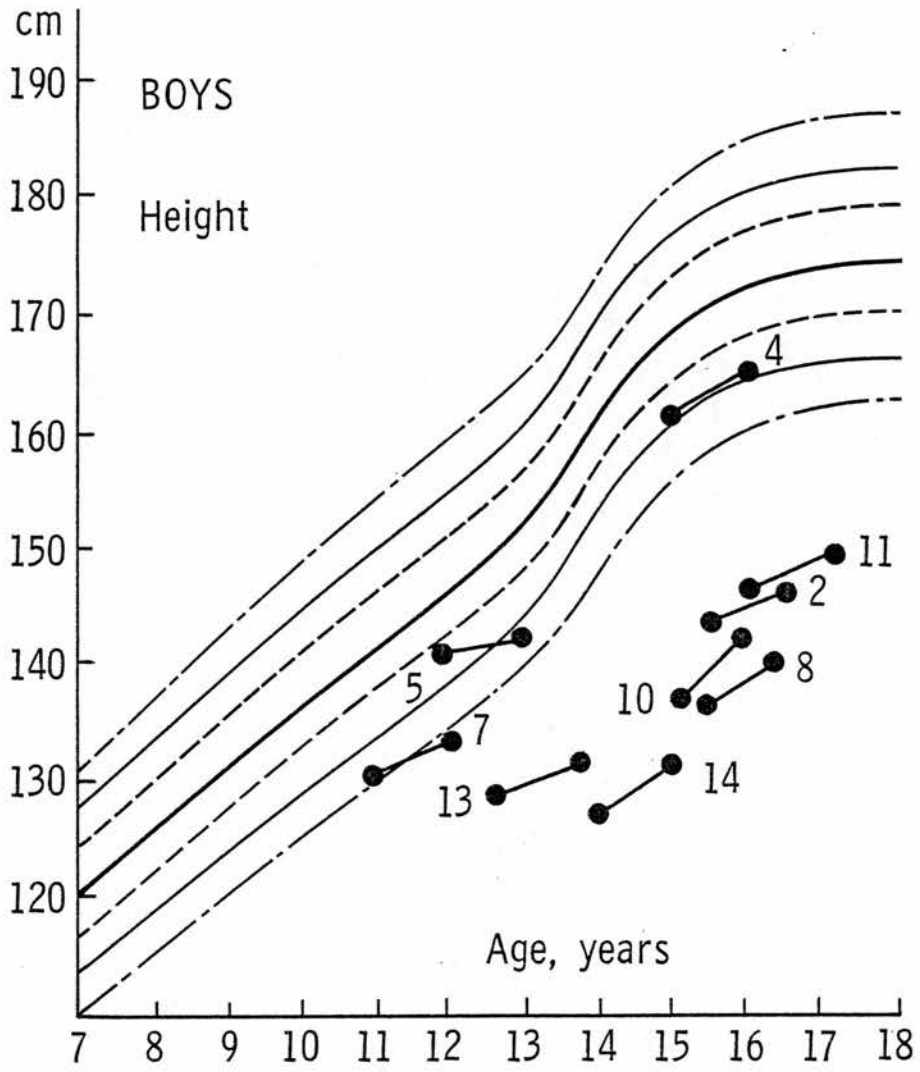

FIG. 15 (a) Height at the beginning and end of study plotted against chronological age of boys.

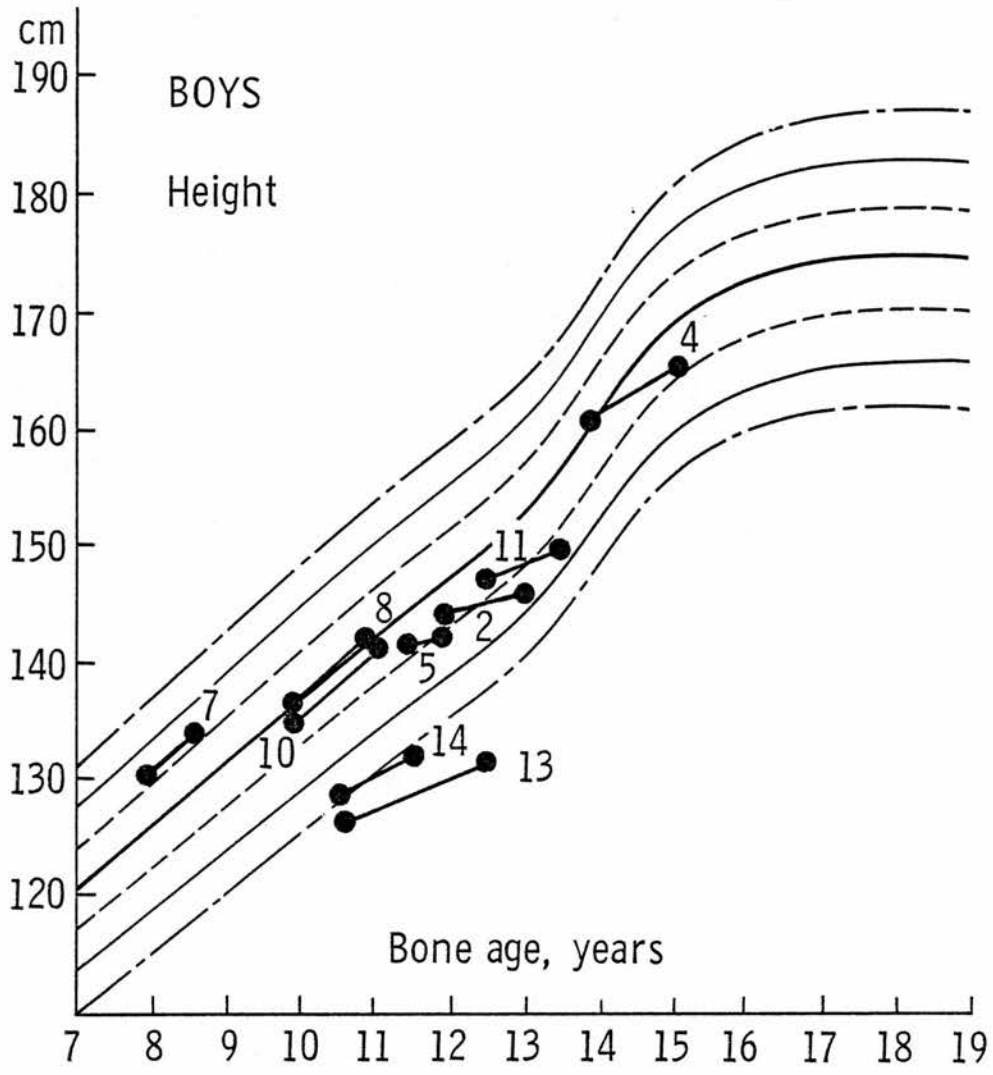

FIG. 15 (b) Height at the beginning and end of study plotted against bone age of boys.

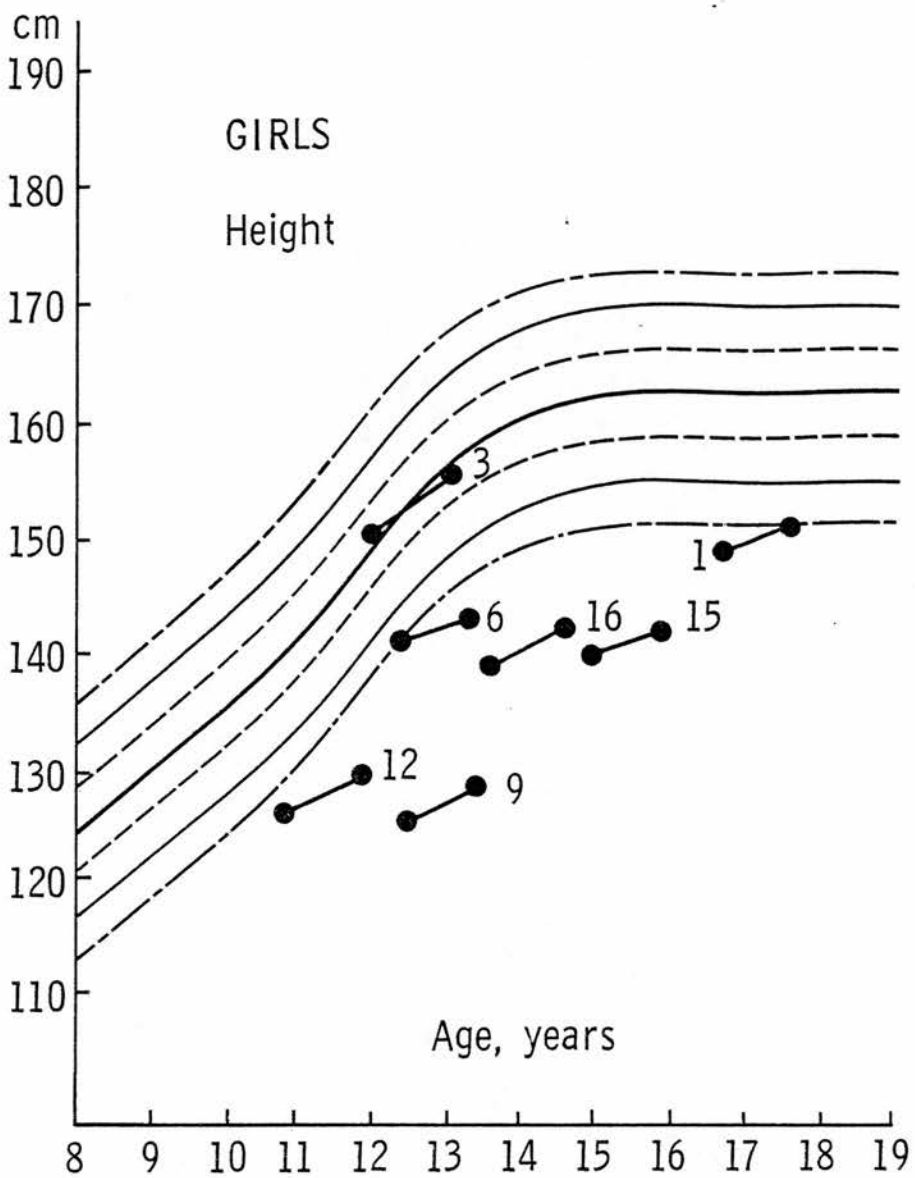

FIG. 16 (a) Height plotted against chronological age of girls.

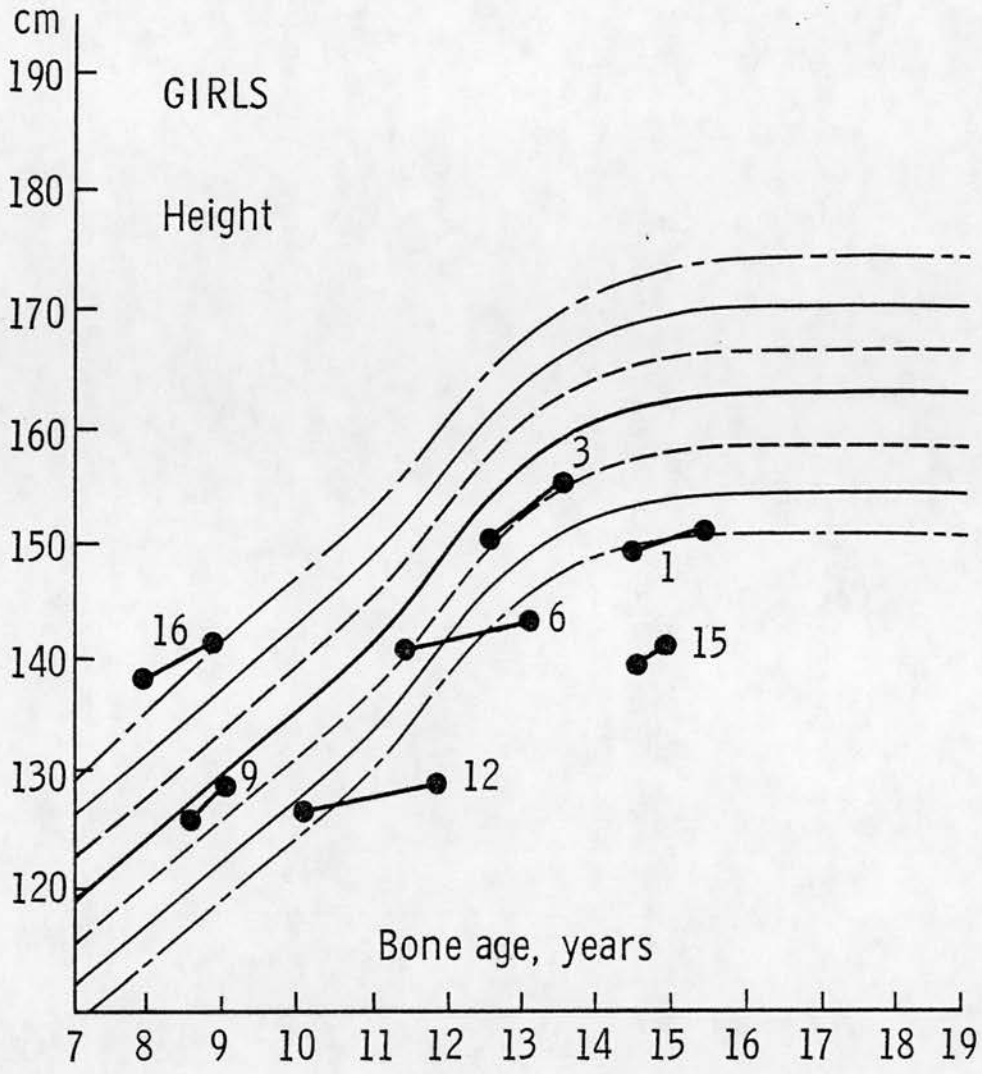

FIG. 16 (b) Height at the beginning and end of study plotted against bone age of girls.

BOYS:

TABLE 25

PATIENT No.	CA (yrs) (M)	BA (yrs) (M)	HEIGHT (cm) S	HEIGHT (cm) M	HEIGHT (cm) E	HSDS CA	HSDS BA	PUBERTY RATING S G	PUBERTY RATING S P	PUBERTY RATING E G	PUBERTY RATING E P
2	16	12.5	143.6	144.1	146.5	-4.31	-0.81	1	1	1	1
4	15.5	14.5	160.1	162.5	164.8	-1.3	-1.09	3	3	4	4
5	12.3	11.5	141.7	142.5	142.6	-1.06	-0.31	1	1	1	1
7	11.5	8	130	131.8	133.8	-1.97	+0.98	1	1	1	1
8	14.8	10	136.6	137.9	140	-4.66	+0.16	1	1	2	1
10	14.6	10	136.2	138.5	141.8	-4.06	+0.26	3	3	4	4
11	15.6	13	146	147.5	148.9	-3.56	-0.71	3	2	3	3
13	14.5	11	127.4	130.6	131.3	-5.26	-1.79	1	1	1	1
14	12.2	10.5	128.7	130	131.5	-2.61	-1.49	2	1	2	1

G Genital Development 1 (Prepubertal) - 5 (Adult)
P Pubic Hair 1 (Prepubertal) - 5 (Adult)

GIRLS:

PATIENT No.	CA (yrs) (M)	BA (yrs) (M)	HEIGHT (cm) S	HEIGHT (cm) M	HEIGHT (cm) E	HSDS CA	HSDS BA	PUBERTY RATING S B	PUBERTY RATING S P	PUBERTY RATING S M	PUBERTY RATING E B	PUBERTY RATING E P	PUBERTY RATING E M
1	17.2	15	149.6	150.1	151	-2	-1.97	5	4	1	5	5	1
3	12.4	13	150.4	153.3	156	-0.05	-0.58	2	3	0	5	5	1
6	12.8	12	141.3	141.7	143	-2.51	-1.3	3	1	0	3	2	0
9	12.8	8.5	125.9	127.5	129.3	-4.2	-0.05	1	1	0	2	1	0
12	11.3	11	127.1	128	129.1	-2.88	-2.3	1	1	0	1	1	0
15	15.4	14.5	139.8	141.1	141.6	-3.5	-3.36	5	4	1	5	5	1
16	14.1	8	139.1	140.3	142	-3.35	2.66	2	2	0	3	2	0

B Breast Change 1 (Prepubertal) - 5 (Adult)
P Pubic Hair 1 (Prepubertal) - 5 (Adult)
M Menarche 0 Absent 1 occurred

TABLE 25: Observed stature and puberty change over the period of study. Puberty rating according to Tanner (1962) and the patients ordered from high to low rating.

S = start; M = middle and E = end of period of study. Chronological and bone ages are those of the child at the mid-period. HSDS = Height Standard Deviation Score.
CA = Chronological Age; BA = Bone Age.

TABLE 26

Patients	Growth velocity cm/year	GVSDS	Growth rating
1	1.4	+0.78	3
2	2.9	-1.41	2
3	5.6	+0.25	3
4	4.7	-0.75	3
5	0.9	-3.07	1
6	1.7	-2.70	1
7	3.8	-2.15	3
8	3.4	-2.15	2
9	3.4	-1.79	2
10	5.6	+0.60	3
11	2.9	-1.64	2
12	2.0	-2.56	1
13	3.9	-1.00	3
14	2.8	-2.55	2
15	1.8	+0.26	3
16	2.9	-2.57	2

TABLE 26: Observed growth velocities, growth velocity standard deviation score (GVSDS) for mid-period bone age and growth rating according to puberty status.

Figures 17 and 18 show the growth velocities of the boys and girls respectively during the period plotted against their mid-period chronological and bone ages.

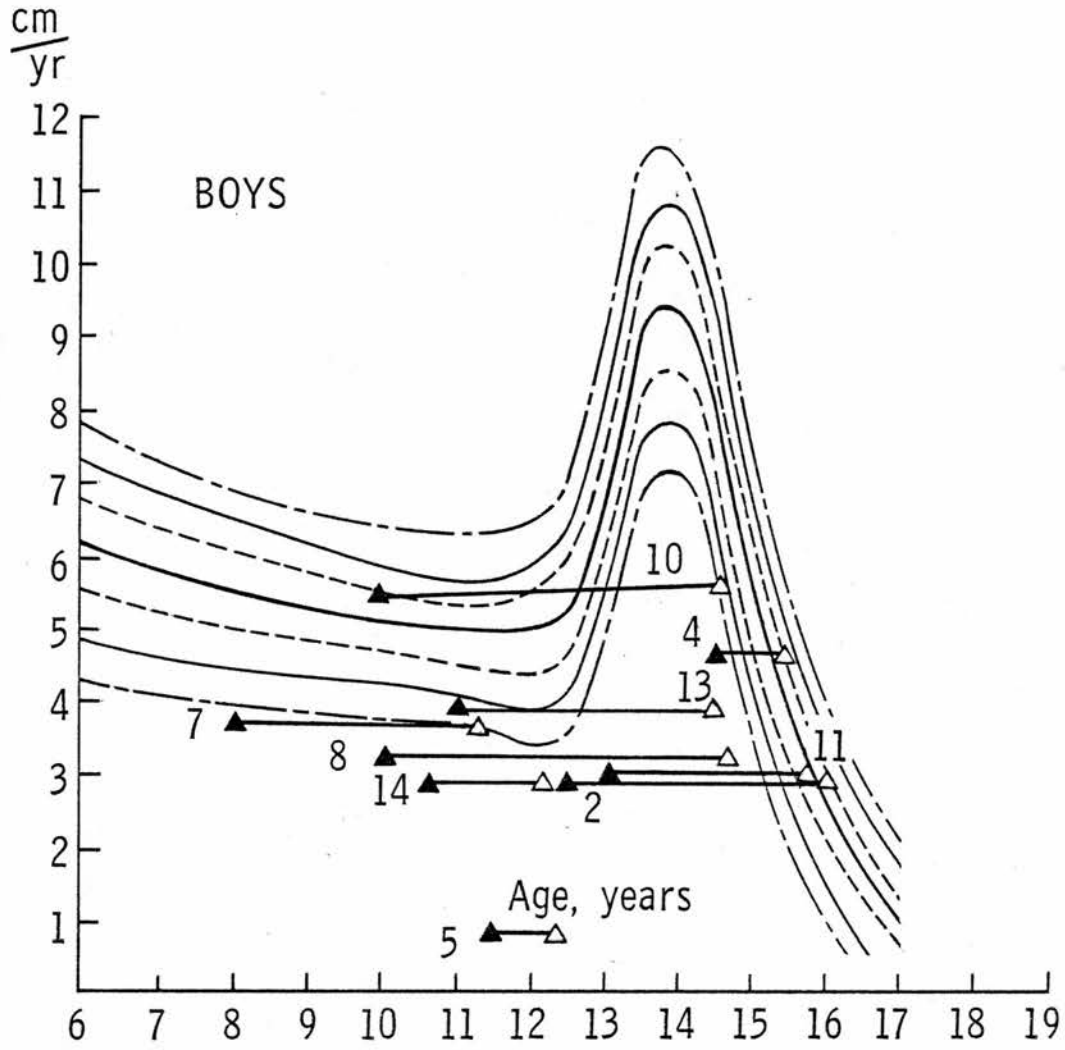

FIG. 17 Growth velocity plotted against chronological age (△) and bone age (▲) of the boys.

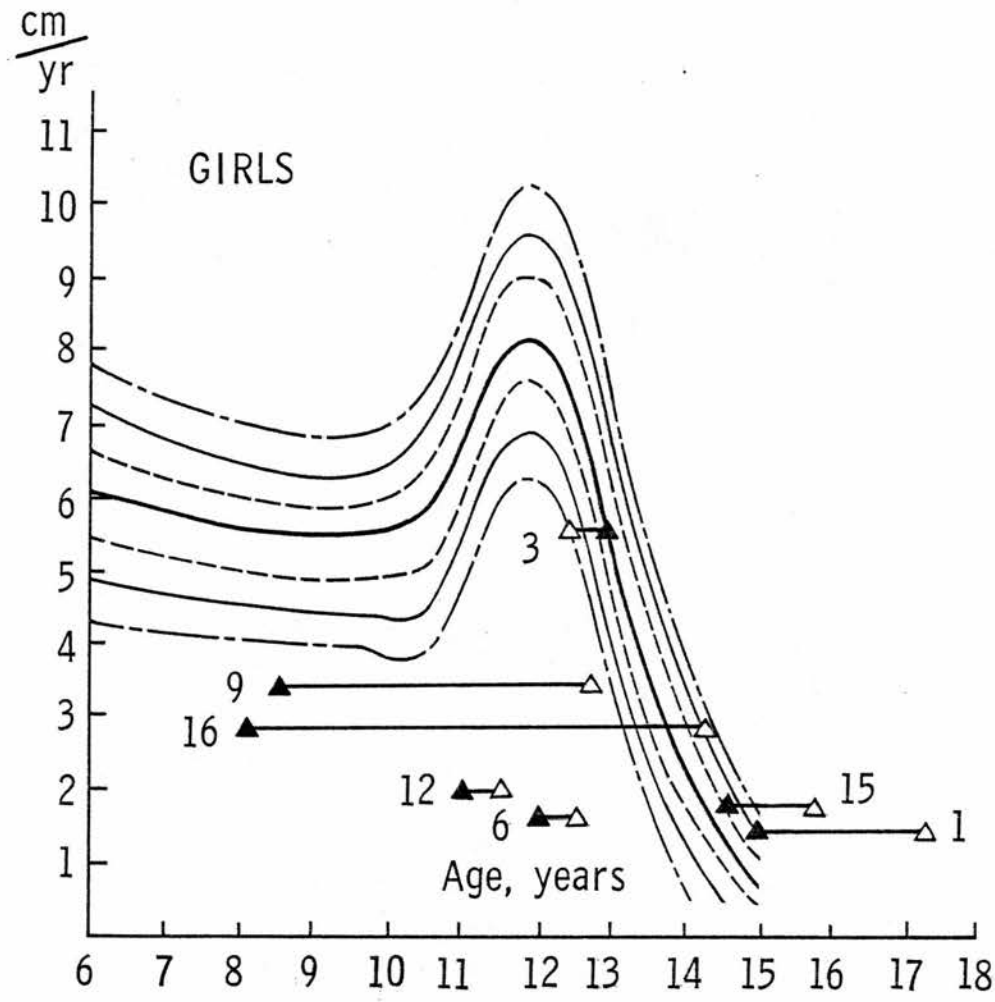

FIG. 18 Growth velocity plotted against chronological age (△) and bone age (▲) of the girls.

Puberty Status

At the beginning of the period of observation five boys (numbers 2, 5, 7, 8, 13) were prepubertal, one of whom (number 8) entered puberty during the period of assessment, and four showed various degrees of sexual development (Table 25). On the other hand only two girls (numbers 9, 12) were prepubertal, one of whom entered puberty during the period of study. The remaining five girls showed various stages of sexual development.

Skeletal maturation

Ten children had a delay of bone age greater than one year and in seven of these the delay was more than three years. On average, the retardation of bone maturation was less severe in girls than in boys, but large individual variations occurred. The degree of delay in bone age was not greater in older children, suggesting that this occurred at an early age and was not necessarily progressive. Figure 19 demonstrates the course of skeletal maturation in the boys and the girls during the period of study.

Stature

Six boys had statures below the third percentile at the beginning of the observation period (numbers 2, 8, 10, 11, 13, 14) and remained consistently below the third percentile during the year, and three boys (numbers 7, 5, 4) were on or above the third percentile, one of whom (number 5) showed a marked fall in height centile at the end of assessment period. Seven had significantly retarded bone ages so that when height was plotted against bone age only one remained below the third percentile. Five girls (numbers 1, 9, 12, 15, 16) had statures

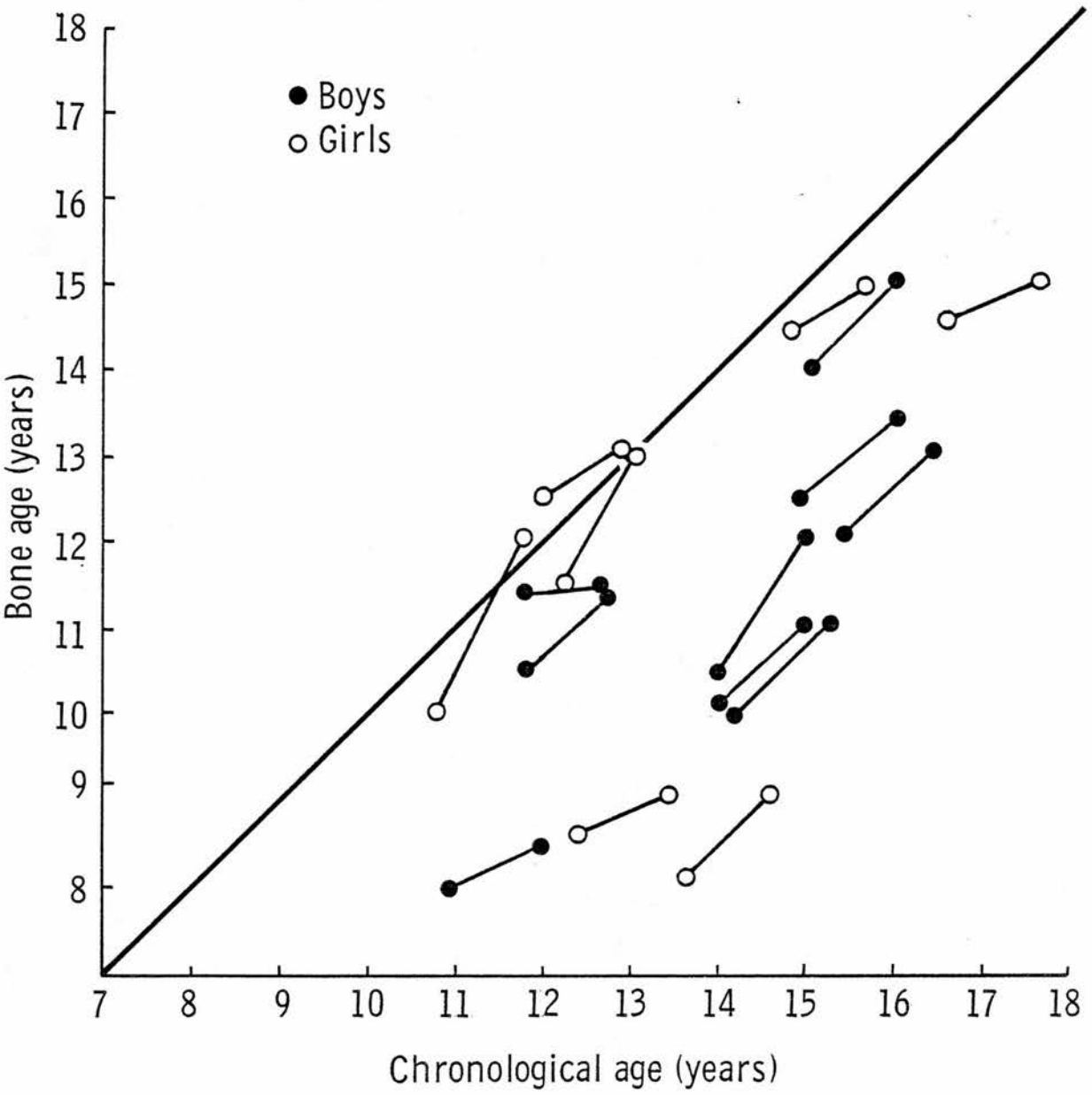

FIG. 19 Correlation of Chronological age with bone age at the beginning and the end of the period of study.

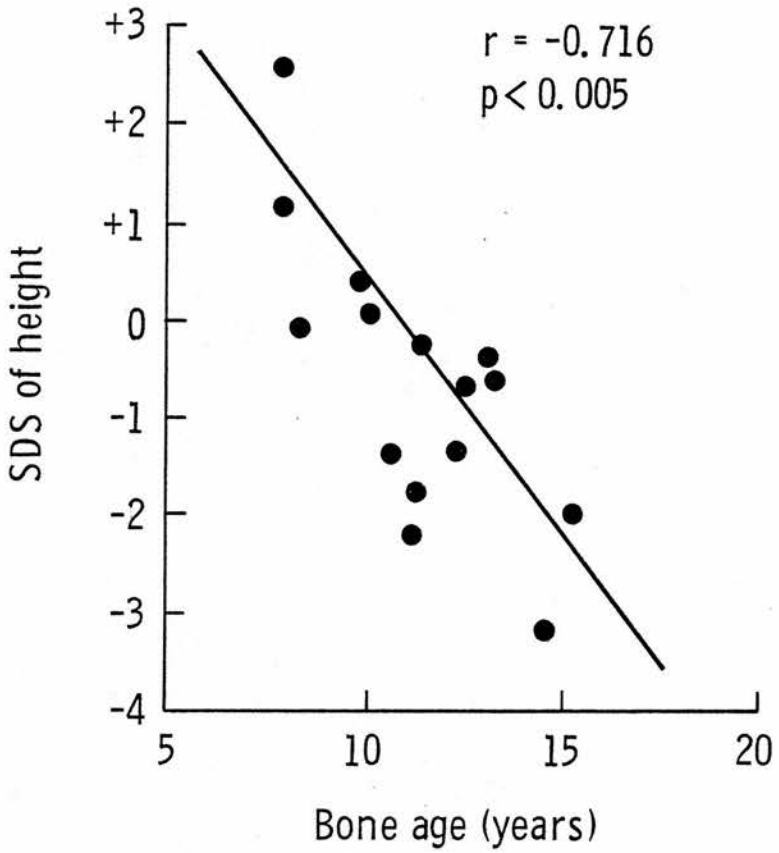

Fig.20. The relation of height, expressed as standard deviation score (SDS), to bone age.

below the third centile at the beginning of observation and, with the exception of patient (1) who reached the third centile at the end of observation, remained below the third centile throughout the year. While patient (number 3) followed the 50th centile throughout the study, patient (number 6) who was on third centile at the beginning, had stature below the third at the end of observation. Three girls had significantly delayed bone ages. When height was related to bone age only two girls were below the third centile. It is apparent from Figures 17, 18 that height gain may continue in older children after it might have been expected to cease in normal children. These children may eventually be taller than expected from their current growth performance though, because of the obvious tendency for statural growth not to increase at the same rate as the advance of bone age, their ultimate stature will be reduced.

There was a significant fall in the height centile of the sixteen children, expressed as standard deviation score for bone age, with increasing bone age ($r = -0.71$; $p =< 0.005$) indicating that the potential for growth falls with increasing age (Figure 20). This point was further clarified when the changes in skeletal maturation were compared with the changes in body height, it was apparent that the increase in bone age during the period of observation was more than the increase in height age.

Body weight

Figures 21, 22 show the mean post-dialysis weight during one month at the start and end of the period of assessment plotted against

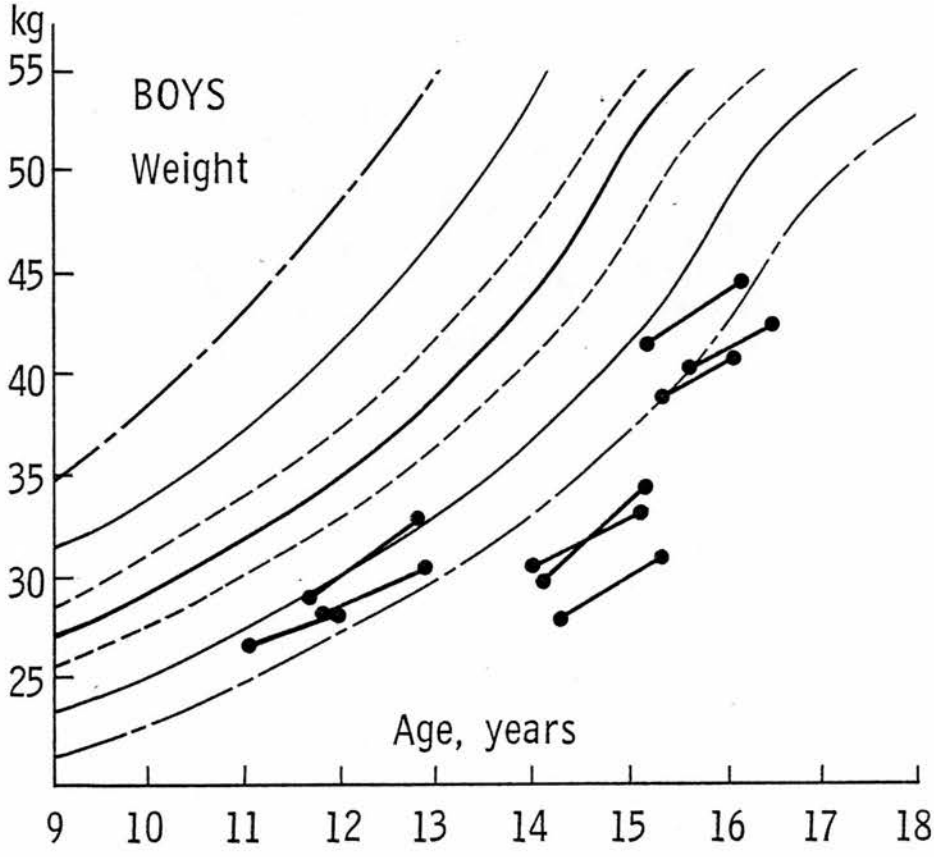

FIG. 21 Mean post-dialysis weight at the beginning and end of study plotted against age (Boys).

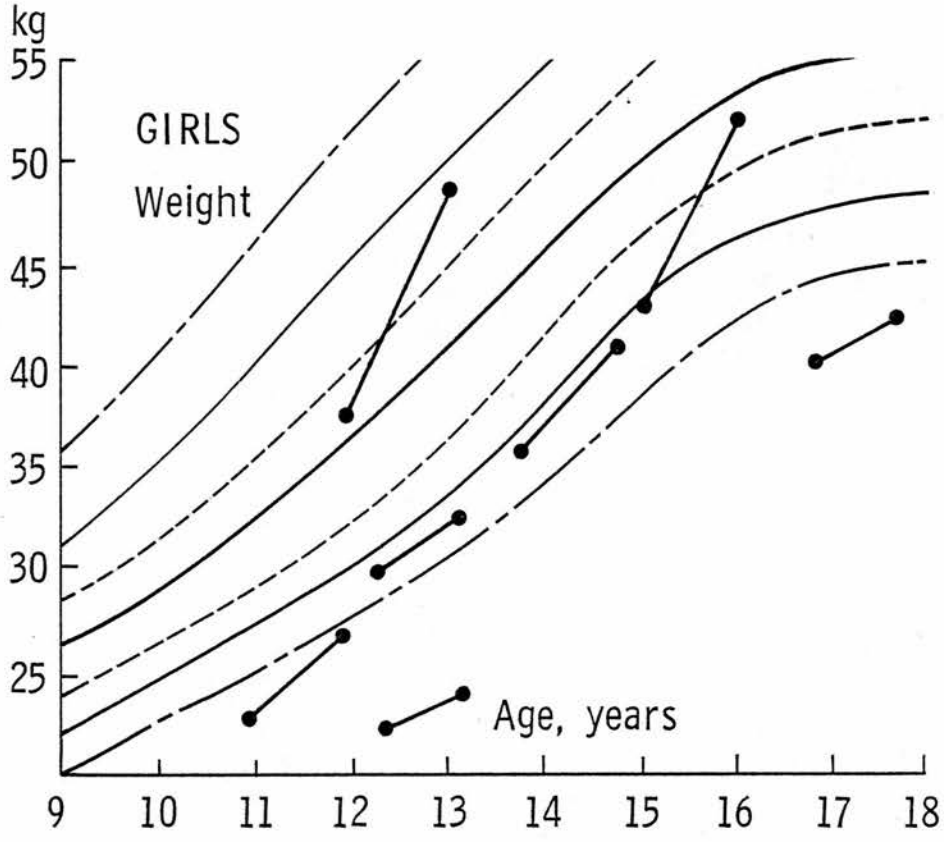

FIG. 22 Mean post-dialysis weight at the beginning and end of study plotted against age (Girls).

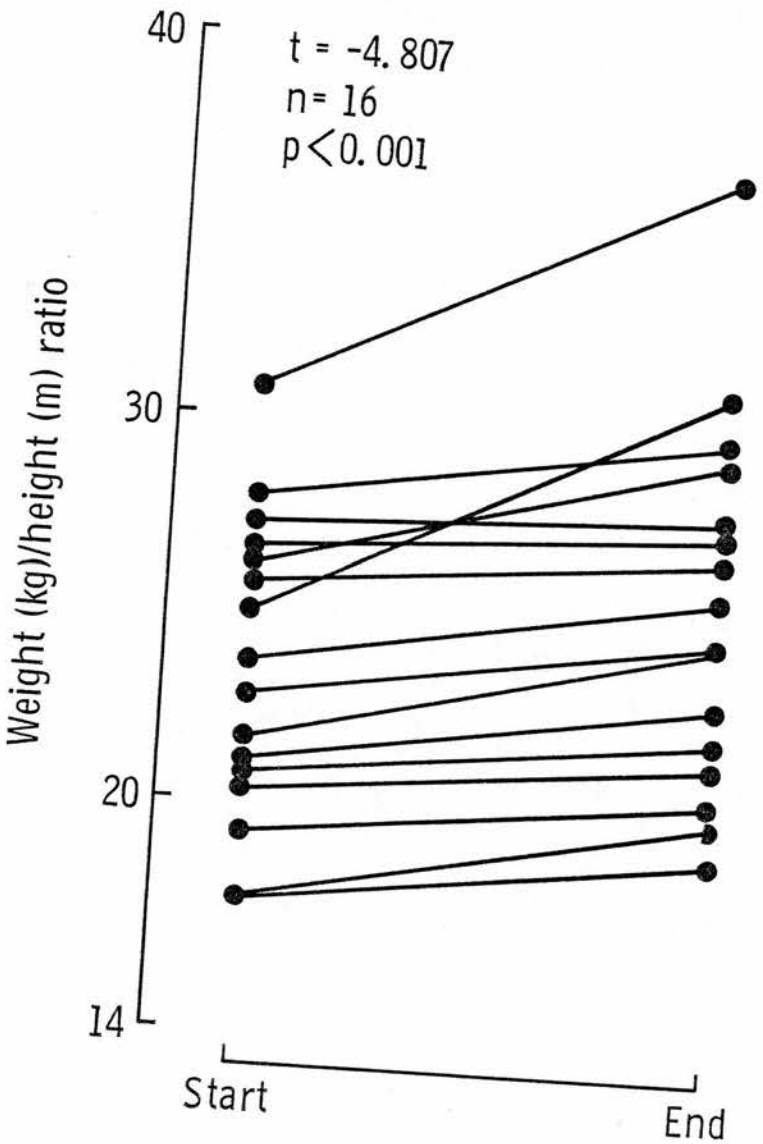

Fig. 23. Body weight (mean post-dialysis for one month) for height ratio at the start and end of period of study (one year).

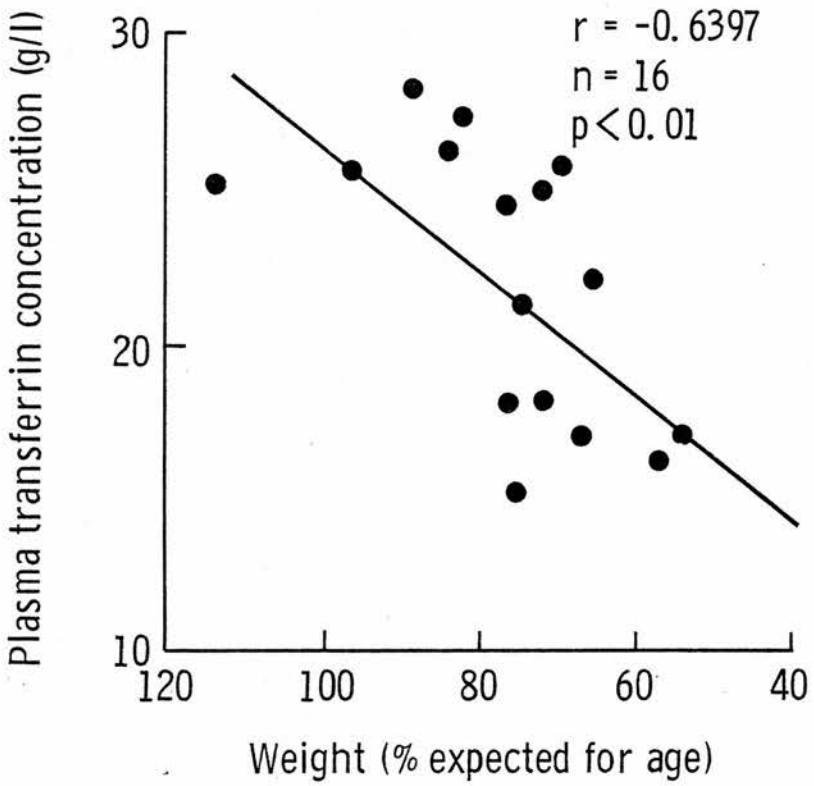

Fig. 24. (a) Relationship between plasma transferrin concentration and weight (% expected for age).

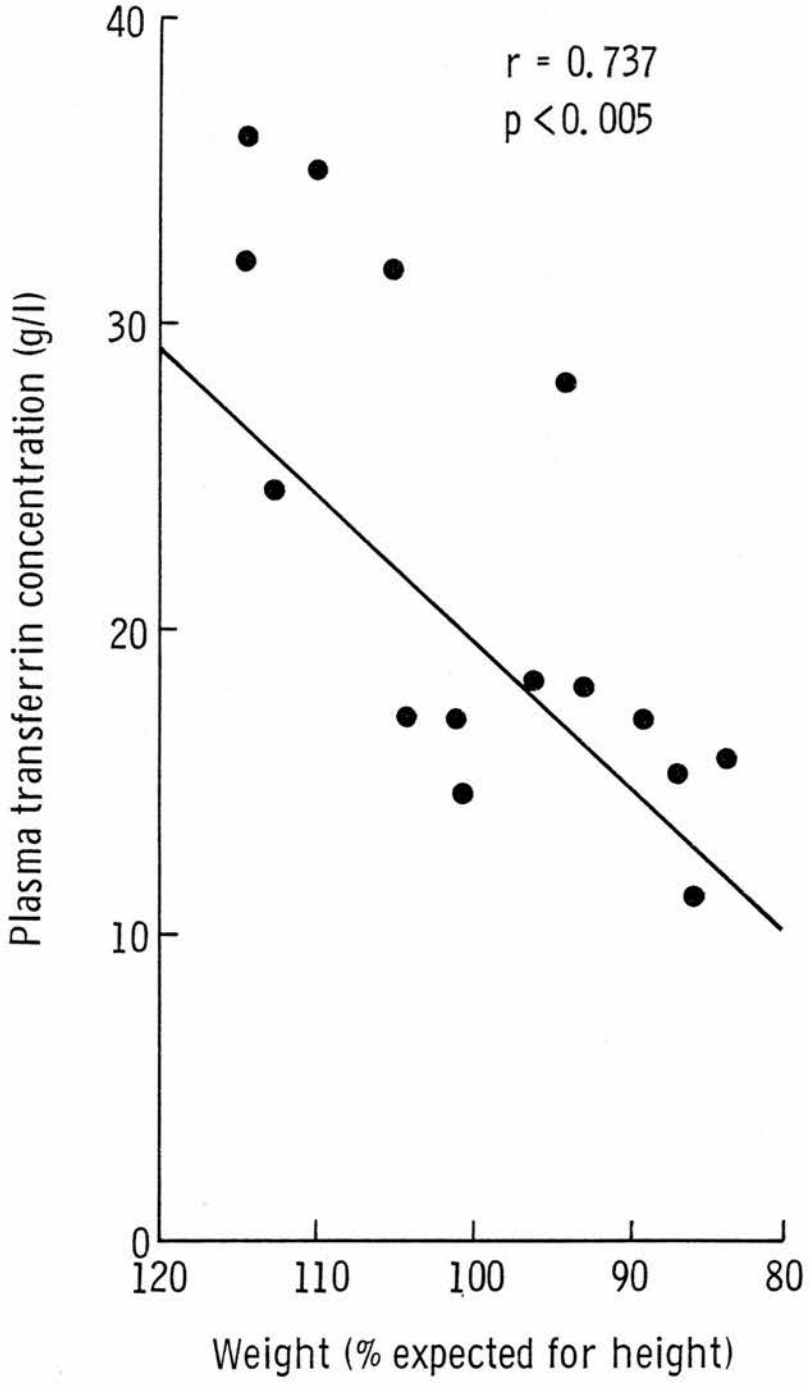

Fig. 24(b) Relationship between plasma transferrin concentration and weight (% expected for height).

chronological age. At the beginning of the study, three boys had weights below the 3rd centile and remained so at the end of the study; six boys were on or above the 3rd centile, 3 of whom showed a fall in weight centile at the end of the period of observation (Figure 21). Four of the girls were above the 3rd centile at the beginning, two of whom had a marked increase in weight centile at the end of the period. Three girls were below the 3rd centile and remained so throughout the study, though one patient (number 12) was just below the 3rd centile at the end of the assessment period (Figure 22).

The weight for height ratio $(\frac{Wt}{Ht})$, calculated for each patient at the start and end of the period of observation, is shown in Figure 23. The increase in weight for height ratio was statistically significant ($P < 0.001$, paired 't' test).

The deficit in weight for age and for height (% expected normal) calculated at the middle of the period, correlated inversely with plasma transferrin concentration (Fig.24a,b) but not with dietary intake of energy or protein.

Height Velocities

Figures 17 and 18 show the growth velocities of the boys and the girls respectively during the period of the study, plotted against their chronological and bone ages. The height velocities (cm/year) were calculated and expressed, as described in the methods, both as standard deviation score for mid-point bone age (GVSDS) and in relation to the child pubertal status (growth grades) (Table 26). Of the five boys who were prepubertal at the beginning of the study (numbers 2, 5, 7, 8, 13)

three had growth rates that were subnormal for prepubertal children and two (numbers 7, 13) had a satisfactory growth rate. Patient number 14 who was in early puberty at the start of study grew inadequately. The remaining three boys (numbers 4, 10, 11) were pubertal at the beginning of the study, two (numbers 4, 10) grew satisfactorily and one (number 11) had inadequate growth. The two prepubertal girls (numbers 9, 12) had poor growth rate and of the five pubertal girls (numbers 1, 3, 6 15, 16) three (numbers 1, 3, 15) had adequate growth and two (numbers 6, 16) showed poor growth for their pubertal status. On the whole, pubertal children had better growth performance than prepubertal children, taking puberty status into account.

Bone disease

Bone disease gradings, as described in the methods, at the start, middle and end of the period of study for each patient are shown in Table 27. At the start of the study there were eight children with no definite evidence of subperiosteal erosions (grade 0), two of whom developed moderate to severe erosions (grades 1-2) at the time of the last observation. Six children had initially grade 2 osteodystrophy four of whom improved over the year and two remained the same.

Clinical and biochemical factors and linear growth

There was no significant relationship between growth velocity, expressed as 'SDS', and the duration or amount of haemodialysis, the diastolic blood pressure, the degree of acidosis, the severity of anaemia, or the plasma urea, creatinine, calcium, phosphate, alkaline phosphatase,

TABLE 27

Patients No.	Renal osteodystrophy grading		
	S	M	E
1 *	2	1	1
2 *	2	1	0
3 *	2	1	1
4 *	2	1	0
5	0	0	0
6	0	0	0
7	0	0	0
8 *	2	2	2
9	0	0	0
10 *	0	1	1
11	0	0	0
12 *	1	0	0
13	0	0	2
14 *	2	2	2
15 *	1	1	2
16	0	0	0

TABLE 27: Bone disease (grading) in the patients as assessed at the start (S), middle (M) and end (E) of period of study.

* Taking dihydrotachysterol (DHT).

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and potassium concentrations (Table 28). Similarly, no significant differences were found in the various clinical and biochemical measurements between patients with adequate growth performance (Grade III) and those with poor growth (Grade I).

TABLE 28

	GVSDS	
	r	p
Duration of dialysis	+0.209	NS
Amount of dialysis	+0.286	NS
Pre-dialysis:		
Urea	+0.118	NS
Creatinine	+0.209	NS
Calcium	+0.127	NS
Phosphate	-0.052	NS
Potassium	+0.361	NS
Bicarbonate	+0.342	NS
Alkaline phosphatase	+0.003	NS
PCV	+0.308	NS
Albumin	+0.135	NS
Transferrin	+0.212	NS
Complement C_3	+0.118	NS
Diastolic BP	-0.410	NS

TABLE 28: Relationship between growth velocity, expressed as standard deviation score (GVSDS), and mean pre-dialysis biochemical measurements. Relationships to the duration and amount of dialysis and to diastolic blood pressure are also included.

Growth and Nutritional Status

The relationship between growth velocity and the mean nutrient intake over the year of study as well as the intakes recorded at the middle of the period of growth observation are shown in Table 29. No statistically significant correlation could be demonstrated. Similarly, plasma albumin , transferrin and the complement protein C3

TABLE 29

		Total energy		Protein	
		1	2	1	2
GVSDS	r	-0.254	-0.10	0.139	-0.009
	p	NS	NS	NS	NS

TABLE 29: Relationship between growth velocity, expressed as standard deviation scores (GVSDS), and diet (as % R.I.)

1 = Intakes recorded at the middle of the period of growth observation.

2 = Mean intake over the year of study.

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concentrations, indices of protein status, did not correlate with linear growth (Table 28).

Growth and Osteodystrophy

The severity of bone disease had no apparent influence on linear growth rate : six patients had a moderate to severe bone disease at the start of study (Table 27), and although the lesion improved in four of them, this was not associated with improvement of growth. Similarly, only three out of the eight patients who had no radiological evidence of osteodystrophy had a satisfactory growth rate (Grade III). Patients, numbers 1, 3, 4 and 15, had satisfactory growth despite the presence of bone disease. Renal osteodystrophy had no effect on bone maturation rate which continued to advance in patients with evidence of bone disease.

TABLE 30

	Insulin		GH		Cortisol		T_4		T_3		SM	
	1	2	1	2	1	2	1	2	1	2	1	-
r	0.168	0.235	0.289	0.179	-0.246	-0.030	-0.355	-0.252	0.07	0.226	0.080	
GVSDS												
p	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

TABLE 30: Relationship between growth velocity, as standard deviation score for bone age (GVSDS), and plasma insulin, growth hormone (GH), cortisol, thyroxine (T_4) and triiodothyronine (T_3) concentrations, and serum somatomedin activity (SM).

1 = Mid- period values.

2 = Mean values over the year of study.

Hormones and Growth

(1) Metabolic hormones:

There was no significant relationship between growth velocity, expressed as SDS, and both the means and mid-period values for plasma growth hormone, insulin, cortisol and somatomedin concentrations. The correlation coefficients are shown in Table 30.

Only mid-period values for plasma somatomedin levels were available for analysis.

(2) Thyroid hormones:

Similarly no significant correlation was found between growth and either plasma T4 or T3 concentrations (Table 30).

(3) Sex hormones:

The relationships between growth and the mean plasma concentration of testosterone, gonadotrophins and prolactin in boys and girls are shown in Table 31.

TABLE 31

GVSDS	Testosterone	LH	FSH	Prolactin
Boys: r	+0.798	+0.797	+0.832	-0.635
p	<0.01	<0.01	<0.001	<0.1
Girls: r	+0.469	+0.875	+0.925	+0.542
p	NS	<0.01	<0.005	NS

TABLE 31: Relationship between growth velocity, as standard deviation for bone age (GVSDS) and mean plasma testosterone, leutinizing hormone (LH), follicular stimulating hormone (FSH) and prolactin levels in boys and girls.

In the boys there was a significant positive correlation between GVSDS and testosterone, LH and FSH ($p < 0.01$, < 0.01 and < 0.001 respectively). Plasma prolactin levels in eight of the boys related inversely to growth velocity although this did not reach significance ($r = -0.6440$, $p < 0.1$). Patient number 8 was excluded from analysis as his plasma prolactin level was determined once at the start of the study and was exceedingly high. In the girls, growth velocity correlated positively with both plasma LH and FSH concentrations ($p < 0.01$ and $p < 0.005$ respectively) but did not relate significantly to either plasma testosterone or prolactin levels.

In order to identify the effect of puberty on growth performance the data was pooled and further analysed according to puberty status of the children (Table 32). Plasma gonadotrophin concentrations correlated positively with growth velocity in pubertal but not in prepubertal patients. Plasma testosterone was higher in the pubertal children with satisfactory growth performance than in those with poor growth. In the prepubertal patients, on the other hand, both plasma testosterone and prolactin concentrations tended to be inversely related to growth velocity but these relationships were not statistically significant.

Growth and metabolic parameters

There was no significant correlation demonstrable between growth velocity and the mean of either blood glucose, plasma triglyceride, cholesterol, non-esterified fatty acids or serum glycerol concentrations.

TABLE 32

GVSDS	Testosterone	LH	FSH	Prolactin
Pubertal:				
Correlation Coefficient(r)	+0.4687	+0.7721	+0.5889	+0.0839
p	< 0.05	< 0.001	< 0.01	NS
Prepubertal:				
Correlation Coefficient(r)	-0.4585	+0.2386	+0.2713	- .4974
p	NS	NS	NS	< 0.1

TABLE 32: Relationship between growth velocity, expressed as standard deviation score for bone age (GVSDS), and plasma testosterone, leutinizing hormone (LH), follicular stimulating hormone (FSH) and prolactin levels in pubertal and prepubertal children. Sex hormone data was pooled for this analysis.

There was only a slight positive correlation of borderline significance between GVSDS and the mean plasma valine concentrations ($r = +0.452$, $p < 0.1$). This relationship, however, was found to be significant when the plasma valine values obtained at the end of the period of growth observation were plotted against growth velocity SDS ($r = +0.6009$; $p < 0.02$) (Figure 25). Likewise, plasma leucine concentrations at the end of the period correlated significantly and positively with GVSDS ($r = +0.5522$; $p < 0.05$), but the relationship between the mean plasma leucine values and GVSDS was not significant ($p < 0.1$).

No other plasma amino acid measured correlated with growth.

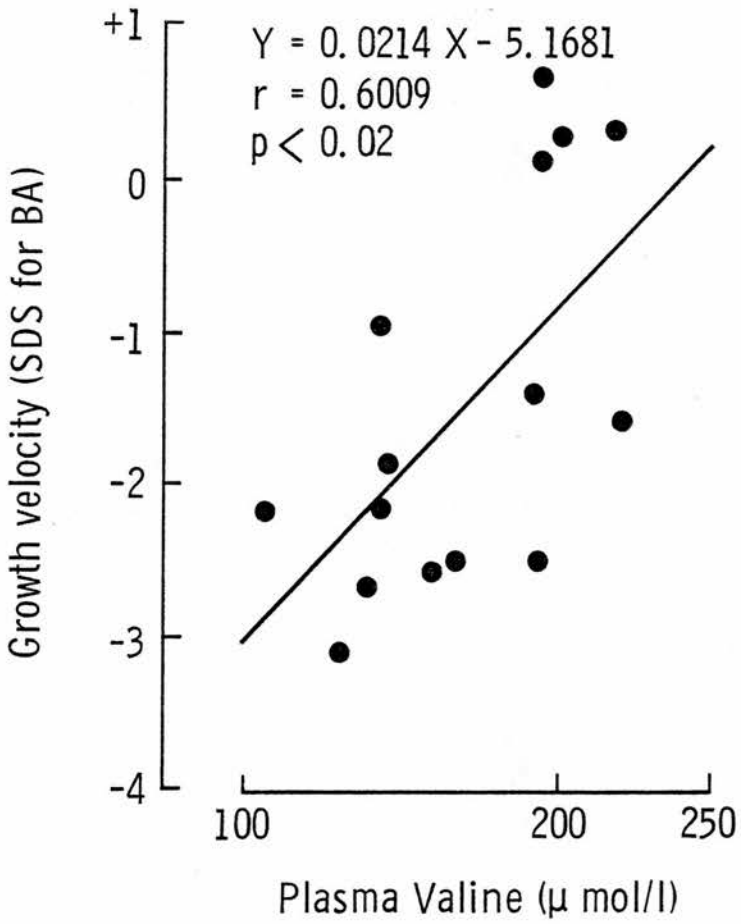

Fig. 25 (a) Relationship between growth velocity (SDS for bone age) and plasma valine concentration.

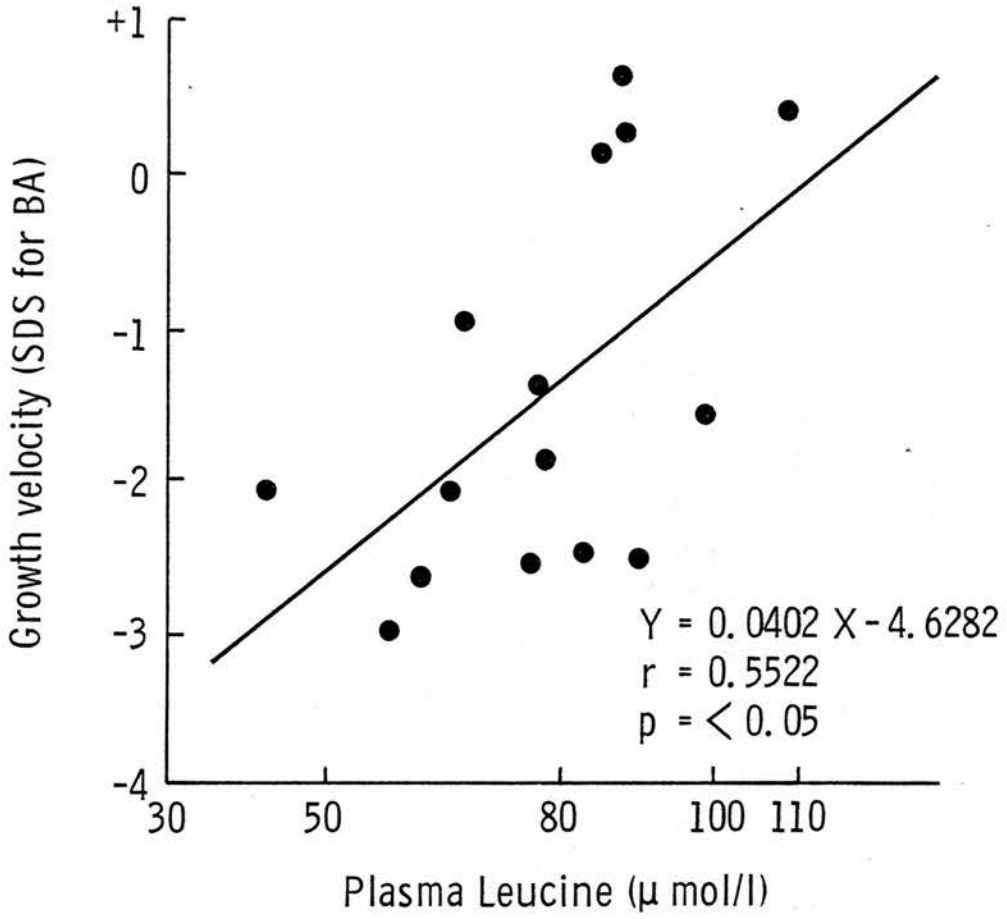

Fig. 25 (b) Relationship between growth velocity (SDS for bone age) and plasma leucine concentration.

DISCUSSION

The purpose of the study was to find out whether the wide variety of metabolic and hormonal disturbances known to take place in adults with renal failure occur in children receiving maintenance haemodialysis. I also hoped to establish clear associations if not causal relationships between the nutritional, metabolic and hormonal changes and to attempt to identify the influences, if any, of such disturbances on growth and development. The present prospective study provides data on linear growth rate as well as the biochemical, nutritional, metabolic and hormonal status of a group of children with renal failure treated by regular haemodialysis at home.

The biochemical data showed that the patients were stable on haemodialysis over the period of study and confirm that regular haemodialysis in the home was technically as successful in children as it is in adult patients. The significantly negative correlation found between the amount of dialysis and both plasma urea and creatinine concentrations might be an indication of the adequacy of dialysis and gives support to the advocation of more frequent dialysis for better rehabilitation of these children (Scharer et al., 1976). However, the adequacy of dialysis was difficult to assess as plasma urea and creatinine concentrations are influenced by the patient's protein intake and muscle mass respectively.

HORMONAL AND METABOLIC CHANGES

The patients in this study were found to manifest a variety of alterations in hormones and substrates involved in carbohydrate, protein and lipid metabolism.

Insulin and Blood Glucose

Peripheral insulin antagonism in chronic renal failure, at least in respect of glucose utilisation, is well documented in adult patients (DeFronzo et al., 1978), and the significantly raised fasting blood glucose in the face of basal hyperinsulinaemia observed in the present study suggests resistance to the hypoglycaemic effect of insulin. As discussed in Chapter 3 carbohydrate intolerance in uraemic adults is more commonly evident on glucose tolerance testing and fasting hyperglycaemia is present only occasionally. The elevated basal blood glucose levels in these children may, therefore, indicate that this abnormality is more pronounced in uraemic children.

The cause of glucose intolerance in uraemic patients is unknown (Chapter 3). A net reduction of glucose utilization could result from impaired peripheral tissue uptake of glucose, augmented hepatic glucose production, or impaired hepatic glucose uptake. Elevated levels of growth hormone have been implicated, but no correlation between growth hormone level and blood glucose concentration could be demonstrated in the present study or by others (Samaan and Freeman, 1970). A significant positive correlation was found between plasma cortisol and blood glucose. This may reflect the well known antagonistic action of cortisol towards insulin both in relation to peripheral cellular uptake of glucose and hepatic glucose production (Munk, 1971).

Although insulin resistance in uraemia appears to be primarily at the periphery (muscle) leading to a decrease in glucose utilization (De Fronzo, 1978), an increase in hepatic gluconeogenesis despite

elevated insulin levels remains a possibility. Dzurik (1973) observed an increase in glucose formation from lactate in liver slices during incubation with uraemic sera, and Frohlich et al. (1974) reported stimulation of gluconeogenesis from amino acids in uraemic rats which was dependent on the presence of the adrenal cortex as adrenalectomy abolished this effect. More recently, Rubenfeld and Garber (1978) demonstrated an increase in gluconeogenesis from alanine with relative impairment of glucose utilization in adult uraemic patients.

The lack of correlation between blood glucose levels and carbohydrate intake would seem to exclude a direct dietary cause for the hyperglycaemia. It was not possible to evaluate the effect of glucose content of the dialysate fluid on the blood glucose levels as all the children dialysed against fluid containing 200 mg/dl of glucose. However, this effect seemed unlikely as the fasting blood glucose levels did not relate to the amount or the duration of dialysis. Daubresse et al., 1976,found that glucose-enriched and glucose-free dialysates had no appreciable influence on fasting blood glucose levels.

Basal hyperinsulinaemia is a common finding in uraemic adults (DeFronzo et al., 1978). This study confirms this and extends this finding to children with chronic renal failure. Decreased degradation of circulating insulin primarily due to loss of renal tissue and possibly also due to reduced hepatic degradation would appear to be a contributary cause of the hyperinsulinism (Chapter 3), but the positive relationship observed in this study between blood glucose concentrations and plasma insulin levels, although not statistically significant, would also

suggest an increased pancreatic insulin secretion in response to the elevated blood glucose levels. The subject is discussed further in the next chapter.

Growth Hormone

Elevated levels of plasma GH have been reported in adults with chronic renal failure (Wright et al., 1968; Orskove and Christensen, 1971). This elevation is due to increased secretion, as shown by the prompt reduction in plasma GH levels following the administration of GH release inhibiting factor (somatostatin) (Pimstone et al., 1975). Like uraemic adults the children in this study had a significantly raised plasma GH concentration. The stimulus for the increased GH secretion in uraemia is unknown. Wright et al. (1968) suggested that protein malnutrition was an important factor determining the increased GH level in uraemia. However, Davidson et al. (1976) found no correlation between GH concentration and dietary protein and I could not demonstrate any correlation between plasma GH concentration and either plasma albumin or transferrin, used as indices of nutritional status, or dietary protein. Plasma GH levels are raised in children suffering from PEM and fall after protein supplementation (Pimstone et al., 1967). Pimstone et al. (1968) originally postulated that it could be the low serum amino acid content which stimulated GH release but this seems unlikely as another important effect of GH is to promote the uptake of amino acids into the tissues and this would decrease further, rather than correct, the serum amino acid concentrations; on the contrary, in normal individuals it is high serum amino acid concentrations which stimulate

GH release rather than the reverse (Raiti and Blizzard, 1970). A direct relationship between basal GH values and fasting plasma NEFA was noted and, since GH is a lipolytic agent (Raben and Hollenberg, 1959), the raised levels in chronic renal failure may possibly be an adaptive phenomenon in an attempt to mobilize endogenous fat for energy production and minimise amino acid catabolism. Glick et al. (1965) suggested that GH secretion may be regulated by the need for a non-carbohydrate source of energy such as fatty acids. Abrupt increases in plasma GH occur in states characterised by actual or impending deficiency of glucose within the intracellular environment (Korner, 1965). If one accept that increase of plasma concentration of branched-chain amino acids towards normal is a reflection of a decrease in amino acid catabolism, then the positive relationships demonstrated in this investigation between plasma GH and the branched-chain amino acids, valine and leucine, might be viewed not as cause and effect relationship but rather as an indirect evidence of a successful adaptation minimising branched-chain amino acids oxidation as energy substrates by increasing the availability of endogenous fat for energy production.

Clearly there is need for more detailed investigation into the stimulus and role of the raised GH in chronic renal failure particularly in relation to energy metabolism.

Cortisol

Little is known about the adreno cortical function in association with chronic renal failure (Chapter 4). Contradictory findings indicating both high (Varghese et al., 1969; Snodgrass et al., 1970) and normal (Lindsay et al., 1969; Betts et al., 1976) plasma cortisol concentrations have been published.

The difference in methods used for plasma cortisol measurements and the difference in the patients studied particularly in regard to degree of renal insufficiency, nutritional status and type of treatment (dialysis or non-dialysis) might account for the discrepancies in results and made direct comparison difficult. Using a more specific competitive protein binding method for plasma cortisol determination (Murphy, 1967), the children in this study had plasma cortisol values I found that although within the upper limit of normal, the mean for the group as a whole was significantly higher than mean control. This is at variance with the finding of Betts et al, using the same method of assay, of normal cortisol levels in a small group of children with varying degrees of renal failure treated conservatively. However, some of their eleven patients had minimal renal insufficiency and only five normal children were tested as controls in their study.

The normal diurnal rhythm of cortisol was however similar to that reported by Betts and his colleagues. The two studies are not strictly comparable since it has been shown that haemodialysis therapy affects plasma cortisol levels (Mishkinet al., 1972; Hrubesch et al., 1973). There is some evidence to suggest that in most dialysis patients cortisol secretion is increased (Mishkin et al., 1972) and that this increase is ACTH-induced. Unfortunately concomitant plasma ACTH levels were not determined in the present study to make any comment on this point. The finding of direct relationship between plasma cortisol and insulin levels was in accordance with the known effect of glucocorticoid inhibition of glucose uptake by cells with simultaneous insulin resistance (Munk, 1971). It has also been

shown (Batstone et al., 1976; Alberti et al., 1975) that in catabolic states cortisol together with other catabolic hormones playsan important role in fuel mobilisation when energy requirements are increased or when the utilisation of glucose as an energy substrate is defective. It was, therefore, possible that the raised plasma cortisol levels were the consequence of insulin resistance rather than the cause of it.

THYROID FUNCTION

As pointed out in Chapter 4, the thyroid status of patients with chronic renal failure remains inconclusive as the interpretation of laboratory findings are particularly difficult due to the complexity of system studied. It is generally accepted that although uraemic patients are clinically euthyroid, low levels of circulating thyroid hormones particularly T3 are common (Lim et al., 1977), and long-term haemodialysis is associated with further decrease in plasma T4 concentration (Dandona et al., 1977).

In the present study, the mean values for plasma T4, FT1 and T3 were at the lower limits of normal. These findings are in agreement with data obtained in uraemic adults (Carter et al., 1974; Lim et al., 1977) and in children with chronic renal failure (Wassner et al., 1977). The reasons for the depressed levels of thyroid hormones remain controversial. They could not be due to a decrease in carrier protein as the thyroxin binding globulin capacity as measured indirectly by T3 resin uptake was normal. Direct measurement of thyroxin binding globulin (TBG) was also reported to be normal in uraemic adults and children (Joasoo et al., 1974; Wassner et al., 1977).

Basal plasma TSH levels, with the exception of the two patients with cystinosis, were not increased. This is generally regarded as evidence against primary hypothyroidism, but hypothalamic-pituitary dysfunction may be present as suggested by the subnormal TSH response to TRH; the cause of which is not clear. Blunted TSH secretion after TRH administration was also reported by Ramirez et al., 1973; Lim et al., 1977 and Czernichow et al., 1976. Silverberg et al. (1973) have also found normal TSH levels with low T4 and free T4 levels in their patients and proposed an abnormal thyroid-pituitary feedback mechanism in renal failure. However, this seems unlikely as the TSH decreases in response to exogenous T3 administration (Czernichow et al., 1976).

According to Carter et al., (1974) and Lim et al. (1977) the peripheral generation of T3 from T4 is decreased in uraemic patients. This may explain the low levels of T3 but does not account for the low T4. Whether the depressed T4 was due to long-term haemodialysis as proposed by Dandona et al. (1977), to a possible intra-thyroidal abnormality as suggested by a subnormal T4 response to exogenous TSH administration reported by Ramirez et al. (1973), to a subnormal pituitary response to TRH, or to a combination of these factors, is not clear.

I have not fully investigated the functional integrity of the thyroid gland in these children, but the findings indicate that they are able to maintain a clinically euthyroid state despite a possible hypothalamic-pituitary dysfunction, and that the subnormal levels of circulating thyroid hormones seemed to have no demonstrable metabolic effects particularly in relation to growth.

The defect in thyroid function in the two patients with cystinosis appeared different from that present in the other children. The depressed plasma T4, FTI and T3 with elevated basal TSH and supranormal TSH response to TRH stimulation are indicative of compensated primary hypothyroidism and intact pituitary-thyroid axis. Similar findings were reported by Chan et al. (1973) and Lucky et al. (1977). The cause is believed to be due to deposition of cystine crystals in the thyroid gland. However, clinical hypothyroidism is uncommon in this group of patients and it is unlikely that the impaired thyroid reserve is the cause of their growth retardation. Thyroxine treatment in seven growth retarded cystinotic children with chronic renal failure resulted in normalisation of basal plasma TSH but had no apparent effect on growth velocity (Burke, El-Bishti et al., 1978).

SEX HORMONES

Little is known about the hypothalamic - pituitary - gonadal axis in patients with chronic renal failure. To my knowledge, sex hormone concentrations and their correlations with the level of sexual development of children and adolescents on regular haemodialysis have not been previously studied.

Plasma LH levels were consistently elevated in virtually all patients in this study and plasma testosterone concentrations were reduced in most of the pubertal boys, a finding in agreement with previous studies in adult male patients (Guevera et al., 1969; Lim and Fang, 1975; Holdsworth et al., 1977). This is consistent with impaired gonadal function with diminished negative feedback at the level of

the hypothalamus-pituitary. The positive linear correlation found between LH and testosterone levels and the reported increased production rate of LH, in conjunction with low testosterone levels, in adult male patients (Holdsworth et al., 1977) supports a role for diminished testosterone feedback in obtaining the elevated LH levels. However, it has been shown (Apostolakis and Loraine, 1969) that gonadotrophins have a renal clearance of 0.4 - 1.7 ml/min, and thus the elevated levels may in part reflect impaired plasma clearance of gonadotrophins.

In few pubertal girls plasma testosterone was increased and was associated with elevated plasma prolactin levels. Raised plasma testosterone is typical of the polycystic ovary syndrome and a proportion of these patients have hyperprolactinaemia (Ginsburg and Havard, 1976). Solitary ovarian cysts occur more frequently in women on regular haemodialysis (Thaysen et al., 1975) but I am unaware of any reports of polycystic ovarian changes.

The number of patients tested with LH/FSH - RH was small for definitive conclusions to be made in regard to the functional integrity of the hypothalamus-pituitary. Nevertheless, the excessive LH response in the pubertal boy could indicate primary Leydig cell dysfunction similar to the findings in patients with other types of testicular damage (de Kretser et al., 1975). On the other hand the blunted initial response with delayed peaks of LH and FSH in the prepubertal and early pubertal boys could possibly be due to some degree of pituitary insensitivity in the younger patients.

The delay in sexual development observed in the patients of this study could, therefore, be the clinical manifestation of gonadal impairment and would be expected to be associated with increased concentration of plasma LH and FSH for the stage of sexual development. The relationship between statural growth and sex hormone levels is discussed below in the section "growth and sex hormone status".

The present study confirms that elevated levels of circulating prolactin occurs in a high percentage of uraemic patients (Nagel et al., 1973; Hagen et al., 1976; Olgaard et al., 1975) and extends this observation to children on regular haemodialysis. The cause of hyperprolactinaemia in chronic renal failure is not known. It may be a result of hypothalamic-pituitary abnormality, a non-specific stress induced response to chronic illness and/or dialysis procedure, or simply related to reduced renal clearance. However, prolactin levels were not related to the duration or amount of dialysis and none of the patients were on drugs which are known to raise the plasma prolactin level.

It has been suggested that raised prolactin levels may have anti-gonadotrophic actions at the gonadal level (Thorner et al., 1974; McNatty et al., 1974). In this respect, the impaired gonadal function in some of the patients in the present study may be related to high prolactin levels. However, the relevance of hyperprolactinaemia to pubertal delay in children is not yet established (Thorner, 1978, personal communication).

NUTRITIONAL STATUS

The nutritional status of patients with chronic renal failure is difficult to quantitate. No overt clinical signs of protein-energy malnutrition were present but delayed bone maturation and retardation of linear growth were common.

Height age rather than body weight was used as reference to recommended intake because young children normally have much higher food intakes in relation to body size compared with older children or adults (Widdowson, 1947). Although the food intake appeared adequate in relation to that of normal children, it is possible that these children's energy requirements might have been greatly increased for catch-up growth to take place as shown to be the case in children recovering from protein energy malnutrition (Ashworth et al., 1968). The biochemical indices of nutritional status - albumin, transferrin and protein complement C_3 were essentially within the normal ranges though there were individual patient variations. Serum levels of transferrin have been described as sensitive indicators of protein deficiency especially when used in studies of Kwashiorkor and correlated well with the severity of protein malnutrition (McFarlane et al., 1969). Low serum transferrin concentrations have been reported in adult patients with chronic renal failure (Ooi et al., 1972; Young and Parsons, 1975) and in these patients it appeared to reflect restricted protein intake (Kluthe et al., 1971; Wardle et al., 1975). In the present study, although there were individual patient variations, the mean for the group was similar to that obtained from controls. This would indicate an adequate protein intake and suggest that measurements of plasma

transferrin concentration are of no value in the assessment of nutritional status of uraemic patients when on an adequate protein intake. However, the value of transferrin for detecting protein-calorie malnutrition in these patients may be limited in the presence of iron deficiency anaemia which causes an increase in transferrin concentration (Mosawe and Rwabwogo - Atenyi, 1973). On the other hand, the lack of correlation between transferrin and protein intake and the presence of significant linear relationship between plasma transferrin and the deficit in weight for age (Figure 24a) are interesting and would suggest that the levels were influenced by protein status of the patients, perhaps as a result of variation in the degree of protein depletion for metabolic and not for dietary reasons. An increase in transferrin catabolism in the patients with the greater body protein depletion would offer an explanation for this relationship. However, studies of transferrin turnover rates in uraemic patients are required to test this possibility.

The results of the present study suggest that plasma transferrin concentrations were not below the normal range until the deficit in weight for age decreased to less than 80% of the standard value.

GROWTH AND DEVELOPMENT

Retardation of growth is common in children with chronic renal failure (Chantler and Holliday, 1973). Most reports on haemodialysed children describe severe growth failure. Poor growth is not, however, inevitable for some children even with severe renal insufficiency whether on or off dialysis grow well (Scharer et al., 1976). Growth

rates of children around the age of puberty with variable degrees of delay in bone age are particularly difficult to assess. For practical reasons most of the children enter haemodialysis programme about that age. The sole use in earlier studies of chronological age in the assessment of growth is invalid because skeletal maturation is frequently delayed in uraemic children. It is important that growth rate should be assessed in relation to bone age because these children may continue to grow after the age when most normal children have stopped growing. It is also clear that growth assessment must take account of pubertal status which is usually delayed in these children. There is as yet no entirely accurate method of assessing pathological growth as the reference growth charts used were compiled for assessing growth in normal children. However, an approximate method frequently used in such situations is the expression of growth velocity as standard deviations for the mean of normal children of comparable bone age.

In order to overcome these difficulties, both standard deviation scores and growth grading, according to puberty status, were used in this study to assess growth performance of the patients. Analysis of growth data in the present study showed that growth rates of most patients were subnormal with variable delay in bone maturation confirming the findings of others. Puberty was delayed in relation to chronological age but, whereas younger children were growing below the third percentile, many adolescent boys and girls were above the normal rate for their chronological age which was undoubtedly due to the delay in bone maturation. In general, pubertal children grew well and prepubertal children did not and no catch-up growth was observed. The

finding of a significant inverse correlation between growth rate and age is unfortunate as it indicates loss of growth potential with advancing bone age. This is similar to the finding of Betts and White (1976) in children with chronic renal failure treated conservatively. As the goal of haemodialysis treatment is successful renal transplantation, satisfactory growth may, therefore, occur in recipients with decreased bone age at operation. The findings of Grushkin and Fine (1973) and Hoda et al. (1975) substantiate this concept. It is important, therefore, that serial determination of bone age should be carried out as an essential aspect of the clinical management of these children and renal transplantation carried out at a bone age of 12 years or less to maximise growth potential.

Various factors have been implicated in the growth failure of uraemic children of which the two most important are renal osteodystrophy and protein-energy malnutrition (see Chapter 2). Although there is no doubt that severe neglected osteodystrophy leads to renal "dwarfism", some children continue to grow despite radiological evidence of bone disease appearing during haemodialysis treatment (Broyer et al., 1974) while others fail to grow inspite of the absence of osteodystrophy. In this study no significant correlation was found between linear growth and the degree of bone disease, and there was no significant difference in the incidence of the bone lesion between children with satisfactory growth grade and those with poor growth. There was also a definite trend towards improvement in bone disease without a comparable improvement in linear growth rate.

In this study no significant correlation between energy intake and growth was found. This is in apparent contradiction to other studies where energy intakes were initially low but growth increased with improved nutrition (Simmons et al., 1971; Betts et al., 1974). Spontaneous energy intake is often reduced in renal failure but the children in this study had been encouraged to eat and were receiving energy supplements during the period of the study and their intakes seemed adequate. Therefore, although better nutrition may improve growth, the provision of extra energy does not ensure it. Betts et al.,(1977) recently reported similar findings. It may be that the energy requirements of these children are in excess of those recommended for normal children of the same size as is the case in children recovering from protein-energy malnutrition; whose energy requirements for catch-up growth is considerably increased (Ashworth et al., 1968). Energy expenditure relates to activity, basal metabolism and growth. While the activity of these children, though not assessed, was unlikely to be increased, an increase in basal metabolism could not be excluded (see Chapter 9). A reduction in the availability of energy for growth was another possibility, and in this respect the finding that some of the children in this study became fatter rather than taller following energy supplementation was interesting and indicated that mcuh of the dietary energy was being deposited as fat and not available for synthetic processes other than those necessary for the formation of adipose tissue. If this is so then it may suggest that fat deficit in the uraemic child is relatively greater than the deficit of lean tissue, and that tissue deposited during energy supplementation has a higher fat content

than it does in a normal child. It may also suggest a defect in energy utilization for lean tissue anabolism perhaps as a result of some other metabolic and/or hormonal disturbances.

One of the major problems in understanding growth failure in these children is how it relates to the hormonal changes described above. A defect in secretion of the anabolic hormone insulin and growth hormone seemed to be excluded as the circulating levels of both of these hormones were increased and no direct relationship was obtained. between growth and either insulin or growth hormone. This, however, does not exclude the involvement of these hormones in the pathogenesis of the growth retardation as their plasma levels may be a reflection of a more fundamental metabolic disturbance consequent upon renal failure and culminating in growth failure.

The lack of correlation between growth velocity and serum somatomedin levels is at variance with data reported by Schwalbe et al. (1977) in a group of children with chronic renal failure treated conservatively and using the same bioassay without correction for plasma sulphate concentration. As it has been shown clearly (Phillips et al., 1978) that sulphate retention in renal failure seriously interferes with the bioassay of somatomedin giving falsely low plasma SM activity, data for serum SM activity in patients with renal failure must obviously be interpreted with caution. However, the finding in this study of positively significant correlation between serum somatomedin activity and plasma transferrin concentration within the group of patients and assuming that sulphate inhibition is the same in all the patients is interesting. It has become apparent that

nutritional status may be a factor that affects the relation between somatomedin and growth hormone. Decreased somatomedin and poor growth despite growth hormone levels that were elevated have been reported in children with protein - malnutrition (Van den Brande et al., 1974; Grant et al., 1973). Serum somatomedin activity correlated positively with plasma albumin and transferrin concentrations, and increased towards normal when the children were refed (Grant et al., 1973).

Chronic renal failure is associated with changes in metabolism of protein qualitatively similar to those of protein-energy malnutrition (Kopple and Swendseid,1975). Plasma somatomedin levels may thus be an indicator of the protein status of patients with chronic renal failure. The finding in the present study of a significant correlation between plasma somatomedin activity and plasma transferrin levels is consistent with this hypothesis. However, the nature of the interaction between growth hormone, nutrition and somatomedin activity is poorly understood.

Phillips et al. (1976) postulated that the decreased somatomedin activity in serum may be associated with either a primary decrease in nutrient supply or a decrease in nutrient utilization for anabolism despite ample nutrient supply such as in diabetes (Van den Brande, 1974). Since insulin deficiency is a feature of both malnutrition and diabetes, it was considered (Phillips et al., 1976) that insulin, and insulin-directed processing of nutrients, are important determinants of somatomedin activity in serum. Since it is recognized that amino acids and glucose enhance the sensitivity of cartilage to stimulation

by somatomedin (Herington et al., 1972), a decrease in nutrient supply in chronic renal failure as a result of malnutrition and/or insulin resistance might conceivably mediate a decreased responsiveness to the growth promoting actions of somatomedin. There is also an experimental evidence (Daughday et al., 1975) to suggest that both insulin and nutrition influence skeletal growth by affecting somatomedin serum level by modulating its hepatic generation.

Growth and sex hormone status

The positively significant relationships found between growth velocity and plasma gonadotrophin levels in both boys and girls, and plasma testosterone concentrations in the boys were obviously a reflection of the effects of puberty on growth as the correlation persisted in the pubertal children and not in the prepubertal children. This indicates that in these children an increase in linear growth rate did occur in response to an increase in sex steroid levels. However, the magnitude of this "adolescent growth spurt" was difficult to assess and one had the impression that although linear growth was accelerated, most of the children did not reach their normal centile for age because of the greater loss of prepubertal growth and the delay in the onset of puberty. The cause(s) of the delay in sexual development was obscure and probably multifactorial. Nutritional causes are important; it is well known that gonadal maturation and function will not take place or be delayed unless the children are adequately nourished and their somatic development reaches a certain critical level. Most of the patients in this study had reduced body size and

weight for age, and, therefore, nutritional factors were likely to be a major cause of the delay in their sexual maturation. Another possible explanation is the interference of uraemia, through unknown mechanisms, with gonadal steroid production in response to circulating gonadotrophin. In male patients with chronic renal failure, decreased testicular volume, normal sex hormone binding protein (SHBG) and a low serum testosterone, which was not altered by gonadotrophin administration have been reported (Stewart - Bently et al., 1974; Holdsworth et al., 1977). The low plasma testosterone with elevated LH found in the boys in the present study was, therefore, suggestive of a defect in testicular steroidogenesis and might be associated with the retardation in their sexual maturation. Most of the children exhibited hyperprolactinaemia and since there is evidence that elevated plasma prolactin levels may act at the gonadal level to block the action of gonadotrophins in both men and women leading to gonadal hypofunction (Thorner and Besser, 1977; Faglia et al.,1977), it is conceivable that the hyperprolactinaemia was causally related to the delay in pubertal maturation of these children. The tendency for the boys with higher prolactin to have the low linear growth rate indirectly supports this view. If this were so then lowering of prolactin levels should result in returning gonadal function to normal, as indeed occurred in female patients on regular haemodialysis treated with bromocriptin (Wass et al., 1978). Obviously the role of hyperprolactinaemia in the delay of sexual maturation of these children and its possible correction warrants further investigations.

Although puberty and the mechanism of initiation of sexual development are still unclear, it can be postulated that they are in

part controlled by the hypothalamic LH - FSH releasing hormone (Roth et al., 1973). The blunted or delayed responses of LH to exogenous LH/FSH - RH in few prepubertal boys tested in this study were indicative of decreased pituitary responsiveness and since the magnitude of LH and possible FSH responses induced by injected LH/FSH - RH seems to be affected by the degree of prior stimulation of the pituitary by endogenous releasing hormone (Roth et al., 1972), it may be postulated that in the boys with chronic renal failure the delay in puberty might be in part, due to hypothalamic - pituitary defect. However, pubertal development is not related to changes in the gonadotrophin secretion alone and must be considered as an integral part of the whole system of hypothalamic, pituitary adrenal and gonadal interactions (Root, 1973).

Growth and plasma amino acids

As will be discussed later, the changes in plasma amino acid concentrations seemed typical of a state of chronic protein catabolism. The positive correlation between growth and certain branched-chain amino acids, though weak and inconstant, was not entirely unexpected and could be interpreted as indicating that as wasting of body protein continued, the supply of certain amino acids such as the branched-chain ones became rate limiting for growth. This does not necessarily mean a causal relationship between linear growth rate and plasma branched -chain amino acid concentrations but rather that growth was adversely affected by the reduction in the availability of essential amino acids for protein synthesis. Indeed, Aronson et al. (1975) have reported a remarkable improvement in growth of a child with severe renal insufficiency following essential amino acids supplementation. The

causes for the accelerated protein breakdown have not been identified. Although under-nutrition could not be excluded, uraemia-induced alterations in energy metabolism might be involved. This latter point required further investigation (see Chapter 8).

PLASMA LIPIDS

Raised plasma triglycerides have been commonly reported in adults on regular haemodialysis (Bagdade et al., 1968: Gutman et al., 1973) and recently Pennisi et al. (1976) and Broyer et al. (1976) reported raised TG levels in children on dialysis. The group of children in the present study showed a similar phenomenon. Type IV hyperlipoproteinaemia is commonly found and the majority of the patients exhibited this pattern. Raised TG levels in chronic renal failure are considered to be due to both increased hepatic TG production (Bagdade et al., 1968; Cramp et al., 1976) and decreased TG clearance from plasma (Cattran et al., 1976; Murase et al., 1975). The positive correlation shown between plasma insulin and TG concentration is similar to that found in uraemic adults (Bagdade, 1970) and it is possible that hyperinsulinaemia stimulates an increased hepatic TG production, as suggested for non-uraemic subjects (Reaven et al., 1967; Bierman and Porte, 1968; Olefsky et al., 1974). The similarities shown between the patterns of change in plasma insulin and those for TG also suggests close association. In uraemic adults an association between triglyceride levels and the degree of glucose intolerance has also been demonstrated (Sorge et al., 1975).

The role of diet in the pathogenesis of hypertriglyceridaemia in chronic renal failure is not clear. No correlation existed between total energy intake and TG concentration, though higher total energy and protein intakes tended to be associated with lower levels. The TG levels found in the patients of this study were about 65% lower than those reported by Broyer et al. (1976) in children on dialysis, and energy and protein intakes in our children were considerably greater than in their study. However, other differences existed in the management of these children, for instance, our patients dialyse for about 30 hours a week compared with 16 hours a week. The proportion of total energy intake derived from carbohydrate correlated significantly with TG concentrations. Both Pennisi et al. (1976) and Broyer et al. (1976) showed a similar relationship between dietary carbohydrate and plasma TG concentration. More recently, Sanfelippo et al. (1977) reported a reduction in plasma TG levels by feeding diet low in carbohydrate and high in polyunsaturated fat. Unfortunately it is not known from their study which of the two variables in the diet altered the TG levels.

Plasma cholesterol concentrations are usually found to be normal in uraemic adults whereas the children in the present study showed considerable increases both compared with controls and with the children reported by Broyer et al. (1976), though 6 of 17 children in the latter study also had significantly increased levels. I was unable to show any relationship between diet and plasma cholesterol, though Broyer et al. suggested a relationship with fat intake. As our patients were taking energy supplements based on food high in saturated fats, it is possible that manipulation of the diet by increasing the polyunsaturated fat

intake might lead to a fall in plasma cholesterol, and this was attempted in the course of this study (see Chapter 10).

Although plasma NEFA concentrations have been reported to fall more markedly in patients with chronic renal failure in response to glucose load (Roth et al., 1973), basal levels were usually found to be within normal limits or reduced (Persson, 1973). Ten of the patients had levels below twice the standard error of the control mean in the presence of elevated plasma insulin and blood glucose concentrations. Intensive haemodialysis lowers plasma insulin concentration and increases insulin degradation rate (Hampers et al., 1970) and there is also evidence that the low NEFA levels rise with adequate dialysis (Tsaltas and Friedman, 1968). The reduced fasting plasma NEFA and low serum glycerol concentration observed in this study might, therefore, be an indication of a relative decrease of endogenous fat mobilization (lipolysis) in uraemia as a direct consequence of basal hyperinsulinism. Insulin is a potent antilipolytic hormone (Cahill, 1971) and also promotes re-esterification of NEFA in adipose tissue, thereby reducing further the quantities of NEFA released into the circulation. However, the possibility of increased hepatic NEFA and glycerol uptake could not be excluded.

PLASMA AMINO ACIDS

The individual amino acid concentrations for the controls were similar to those previously reported from normal children (Armstrong and Stave, 1973). Plasma amino acid concentrations in children are lower than those in adults and increase with maturity (Scriver et al.,

1971). There is, however, no evidence that significant changes occur over the age range of the patients in this study who were, for practical reasons, in the upper age range of childhood, and the controls were of a comparable age and sex distribution.

The alterations in plasma amino acid concentrations were in general similar to those previously found in adults with chronic renal failure (McGale et al., 1972; Young and Parsons, 1970). There are no published reports of plasma amino acids in uraemic children on regular haemodialysis. The main abnormalities consisted of a reduction in the branched-chain amino acids - valine, leucine and isoleucine - and tyrosine and lysine, with a rise in the non-essential amino acids, glycine and alanine. Histidine concentration has been reported to be normal or decreased (Young and Parsons, 1970; Gulyassy et al., 1970).

The reasons for these altered plasma amino acid concentrations are poorly understood. Previous reports have indicated the similarity between the plasma amino acid concentrations seen in uraemia and those found in children with protein-energy malnutrition (Holt et al., 1963), or adults with severe protein restriction (Swendseid et al., 1968). In the present study overt clinical signs of malnutrition were not present but growth retardation and delayed bone maturation frequently were. Dietary intakes expressed according to height age seemed adequate and the biochemical indices of nutritional status were within normal range. While no amino acid correlated significantly with the protein intake, plasma glycine related inversely to energy intake and also tended to vary inversely with protein intake. In children

suffering from protein malnutrition, plasma valine level is a function of dietary protein intake. The distinct lack of correlation of valine with diet in this study suggested that its alteration was due more to effects of the uraemic state on protein metabolism. The plasma albumin concentration also did not correlate with diet and possibly its positive relationship with plasma valine indicates that both reflect the protein status of the individual whether there is protein deficiency for metabolic or dietary reasons. The plasma glycine, on the other hand, may be more responsive to alteration in the actual dietary intake.

The efficacy of dialysis is difficult to measure. All children were dialysed similarly for 30 hours each week with membrane of appropriate size to body surface area and so the plasma urea was probably influenced more by protein intake or endogenous protein catabolism. Both plasma glycine and alanine concentrations showed a significant inverse relationship with plasma urea, but no amino acid correlated with plasma creatinine concentration. It is, therefore, possible that the change in these two amino acids were independent of the efficacy of the dialysis procedure (as evidenced by the plasma creatinine) but that they may have been influenced by other factors which could elevate the plasma urea, such as high protein intake or endogenous protein catabolism. Kopple and Swendseid (1975) have shown that the fall in valine/glycine ratio in patients on regular haemodialysis, whilst mimicked by a low protein diet, was much greater in uraemics in spite of normal energy intake.

That abnormalities in plasma amino acids, typical of protein energy malnutrition, may be seen in uraemic children consuming protein intakes above recommended allowances suggests that these children may have different protein requirements or else that the uraemic state may lead in some way to a state of chronic protein depletion in spite of apparent good food intake. Essential amino acid losses in the dialysate are not greater than the non-essential amino acid losses and are easily replaced by the diet (McGale et al., 1972).

In chronic catabolic states the branched-chain amino acids are reduced (Baertl et al., 1974; Ruderman, 1975). This reduction is considered to be a result of increased muscle protein breakdown and preferential oxidation of the branched-chain amino acids for energy production with increased flux of non-essential amino acids including alanine and glycine into the circulation. Thus alanine formation and branched-chain amino acids oxidation may be directly related with alanine acting principally to remove the amino group from the deamination of the branched-chain amino acids preliminary to their subsequent oxidation (Garber et al., 1976).

Because the uraemic patient may have a decreased ability to utilize conventional sources of energy, it is conceivable that tissues such as muscle continue to utilize branched-chain amino acids as fuel source resulting in an increased production of alanine and a decreased release of the branched-chain amino acids. This may help explain the observation made in the present study of the reciprocal changes in plasma valine and leucine concentration and plasma alanine levels. The similarities in these patients' aminograms to those seen in protein-

energy malnutrition may, therefore, be a result of protein deficiency occurring for metabolic rather than dietary reasons.

It has been postulated that the increase in protein breakdown and the use of muscle tissue amino acids as a fuel substrate is a consequence of lack of insulin or resistance to the action of insulin together with increased levels of catabolic hormones (Cahil et al., 1972; Felig, 1975; Ruderman, 1975). The next study (Study II) was undertaken to define further the hormone-fuel interrelationships in these patients.

CHAPTER 8

STUDY II

HORMONAL AND METABOLIC RESPONSES TO INTRAVENOUS GLUCOSE.

In the preceding study various abnormalities of the metabolic hormones and energy substrates were observed. Most of the children had poor growth despite apparently adequate supplies of dietary energy and protein and were, therefore, insufficiently anabolic. The increased catabolic responses to stress in uraemic rats (Holliday et al., 1977) and the possibility of increased energy and protein requirements for anabolism in acutely uraemic children (Abitbol and Holliday, 1976) suggest the possibility of defective energy utilization for anabolism.

To characterise further the alterations in metabolic hormones and energy substrates, and to better understand the mechanisms governing substrate utilization, the responses of the major fuel substrates, glucose, non esterified fatty acids (NEFA) and amino acids (AA) and the more important metabolic hormones, insulin (IRI), growth hormone (GH) and glucagon (IRG) following intravenous glucose, were investigated in the same group of patients as in "Study I" and compared to values obtained from a group of healthy children.

MATERIALS AND METHODS

Patients and controls

The same patients described in Study I were studied. All were stable at the time of investigation and had been on dialysis for at least six months (range 0.58 - 5.6 years). For ethical considerations entirely healthy children could not be tested. Nine children, five boys and

four girls with a mean age of 12.5 years (range 10 - 14.6 years) being investigated for short stature which ultimately proved to be familial were used as controls ; none of them had renal disease or any other systemic disturbance or a known family history of diabetes. None of them was socio-economically deprived and all were well nourished and in good health at the time of the investigation.

Consent was obtained after full explanation of the test to parents and children and the study was approved by Guy's Hospital Medical School Ethical Committee.

Procedure and methods

Since there is doubt about the effect of chronic renal failure on intestinal absorption of nutrients, the proportion of an oral load of glucose which reaches the circulation is unknown. Nor is anything known of the effects of uraemia on intestinal factors which promote the release of insulin. For these reasons the intravenous glucose tolerance test (IVGTT) was used.

The patients and controls were admitted to hospital for the study and the tests were performed in the morning following an overnight fast of at least twelve hours, and in the case of the patients at least 28 hours post dialysis.

I V G T T

After fifteen minutes rest period in the recumbent position 0.5 g of glucose per kilogram body-weight was administered as a 50 per cent solution over a period of three minutes through a butterfly needle

inserted in cubital vein. Blood samples were taken through another needle placed in the arterio-venous fistula or peripheral vein in the opposite arm at 0, 5, 10, 15, 20, 30, 40, 50, 60 and 90 minutes with the child remaining in bed throughout the test. The sampling needle was kept patent with the use of slow saline drip.

Sample preparations and analytical methods for BG, IRI, GH, IRG, NEFA and AA were as described in "Methods" in Study I and in "Analytical Methods" in Chapter 6.

After the rapid intravenous injection of glucose, the logarithm of the blood glucose level declines linearly with time and the slope of this line, expressed as the functional delay constant K, was used as an index of glucose tolerance. K was calculated by the method of least squares after logarithmic transformation of blood glucose concentration and was multiplied by 100. Thus K x 100 represents the glucose assimilation rate in per cent per minute (Lundback, 1962). Glucose disappearance rate of less than 1.2 was taken to indicate glucose intolerance (Franckson et al., 1962). The early insulin response (0 - 10 minutes) was calculated by substracting the fasting value from the mean of the 5 and 10 minute values. The mean of the absolute values between 20 and 60 minutes following glucose injection was taken to represent the late insulin response (DeFronzo, 1973). The molar ratio of insulin to glucagon was calculated by multiplying

$$\frac{\text{plasma insulin } (\mu u/ml)}{\text{plasma glucagon } (pg/ml)}$$

by 23.33, which is based on 6000 molecular

weight and 25 U per milligram biological activity for insulin and 3500 molecular weight for glucagon (Anthony and Faloona, 1974).

Student 't' test was used to compare results in patients and controls. The significants of changes in response to glucose was assessed by paired t-test.

RESULTS

Detailed clinical data of patients have already been shown (Study I, Table I). Clinical data on the control group are shown in Table 3 of Study I. The results of IVGTT in patients and controls are presented in Tables 1, 2 and Figures 1, 2, 4, 5, 6. Individual patient results are presented in Appendix B.

Blood glucose: Fasting blood glucose levels were significantly raised in the patients compared with controls ($P < 0.001$). Figure I illustrates the blood glucose response in the patients and controls after intravenous glucose administration. In the normal subjects the glucose disappearance (K_G) ranged from 1.49 to 2.52 with a mean of 1.84 per cent per minute. The mean K_G for the patients was 1.09% /min. (range 0.46 to 1.51). This difference was significant ($P < 0.001$) (Table 3). Ten of the patients (62.5%) had K_G values less than 1.2, the reported lower limit of normal glucose tolerance (Franckson et al., 1962).

The degree of glucose intolerance did not correlate with clinical parameters such as per cent ideal body weight, diet, blood pressure and duration of dialysis. Nor was there any relationship with the plasma calcium, potassium, bicarbonate, urea or creatinine concentrations.

Plasma insulin: Insulin responses to i.v. glucose are shown in Table I and Figure 2. The fasting plasma insulin levels were significantly elevated in the patients. Following glucose injection, the early insulin response (0 - 10 minutes) was similar in patients and controls but the late response (20 - 60 minutes) was significantly raised in the patients compared with controls (Table 3). The patients

TABLE 1

Time (Minutes)	0	5	10	15	20	30	40	50	60	90
BG (mmol/l)										
Patients(16)	5.21 ± 0.6	11.77 ± 1.39	10.13 ± 1.14	8.87 ± 1.13	7.84 ± 1.24	6.62 ± 1.25	5.84 ± 1.19	5.29 ± 0.96	5.08 ± 0.91	5.1 ± 0.68
Controls(9)	4.38 ± 0.65	10.75 ± 1.08	9.44 ± 1.08	8.17 ± 1.06	7.17 ± 0.65	5.96 ± 0.82	4.88 ± 0.8	4.08 ± 0.65	3.45 ± 0.41	3.77 ± 0.37
P	<0.001	<0.05	NS	NS	NS	NS	<0.05	<0.005	<0.001	<0.001
IRI (mU/l)										
Patients(16)	16.68 ± 7.7	60 ± 24.8	39.9 ± 13.7	36.7 ± 17.3	33:9 ± 15.7	27.7 ± 15.2	24.4 ± 14.8	21.7 ± 12.7	21.7 ± 7.9	19.9 ± 9.5
Controls(9)	8.2 ± 3.7	36.4 ± 9.2	29.3 ± 14.3	20 ± 8.9	15.4 ± 3.7	14.2 ± 7.1	10.6 ± 3.7	8.1 ± 2.4	6.2 ± 2.5	6.2 ± 2.7
P	<0.01	<0.02	<0.1	<0.02	<0.005	<0.02	<0.02	<0.005	<0.001	<0.001
GH (mU/l)										
Patients(16)	18.25 ± 12.2	24.4 ± 21.9	25.6 ± 26.4	26.8 ± 27.2	29 ± 28.1	30.9 ± 31.5	28.1 ± 27.9	25.8 ± 26.5	21.1 ± 21.4	16.5 ± 11.7
Controls(9)	7.0 ± 4.3	13.4 ± 8.8	11.1 ± 5.4	10.1 ± 5.2	9.0 ± 4.8	7 ± 5.2	4.2 ± 2.7	5.5 ± 3.4	6.4 ± 5.5	11.4 ± 12.5
P	<0.02	NS	NS	<0.1	<0.05	<0.05	<0.02	<0.05	<0.1	NS
IRG (pmol/l)										
Patients(10)	20.3 ± 17.7	-	21.9 ± 12.8	-	-	18.1 ± 9.4	-	-	19 ± 10.3	22.1 ± 10.9
Controls(5)	6.8 ± 4.2	-	5.8 ± 3.7	-	4.8 ± 3.3	-	3.8 ± 1.7	-	1.6 ± 0.89	
P	<0.005		<0.01						<0.005	
NEFA (µeq/l)										
Patients(15)	685.2 ± 97.1	-	-	-	394.4 ± 76.1	-	275.6 ± 65.8	-	314.1 ± 47.9	-
Controls(9)	859.4 ± 113.1	-	-	-	554.8 ± 96	-	459.5 ± 64.6	-	556.8 ± 49.8	-
P	<0.001				<0.001		<0.001		<0.001	

TABLE 1: Results of intravenous glucose tolerance tests (IVGTT)

Values are means (± 1 SD).

BG = Blood Glucose;
IRI = Immunoreactive Insulin;
GH = Growth Hormone;
IRG= Immunoreactive Glucagon;
NEFA = Non-Esterified Fatty Acids.

Figures in brackets indicate number of subjects. Significance of difference (P) was tested by student t-test for unpaired data.

Plasma amino acid (μmol/l)	PATIENTS						CONTROLS					
	Fasting			60 min after glucose		p	Fasting			60 min after glucose		p
	No	Mean	SEM	Mean	SEM		No	Mean	SEM	Mean	SEM	
serine	13	84	6	75	4	<0.05	6	136	12	123	11	NS
citrulline	13	92	4	84	4	NS	6	42	3	37	2	NS
glycine	12	392	30	385	28	NS	6	215	9	196	8	<0.05
alanine	13	337	39	298	27	<0.05	6	308	30	279	26	<0.05
valine	13	164	10	146	9	<0.001	6	217	6	190	13	<0.05
methionine	9	11	2	9	1	NS	4	14	1	15	1	NS
isoleucine	13	44	3	37	3	<0.01	6	56	4	46	4	<0.01
leucine	13	73	5	62	4	<0.001	6	102	5	85	6	<0.01
tyrosine	13	21	1	17	1	<0.01	6	47	3	38	2	<0.05
phenylalanine	13	38	2	38	2	NS	6	41	2	37	3	NS
histidine	13	69	5	69	4	NS	6	90	7	79	3	NS
lysine	11	159	10	148	9	<0.05	6	157	8	141	11	NS
arginine	13	75	5	76	5	NS	5	70	7	60	4	NS

TABLE 2: Plasma amino acids fasting and post-glucose in patients and controls. SEM = standard error of the mean; NS = not significant.

TABLE 3

	PLASMA INSULIN CONCENTRATION			INSULIN/GLUCOSE RATIO			K VALUE FOR GLUCOSE
	FASTING	EARLY RESPONSE (0-10 min)	LATE RESPONSE (20-60 min)	FASTING	EARLY RESPONSE (0-10 min)	LATE RESPONSE (20-60 min)	
PATIENTS (16)	16.7 ± 7.7	33.2 ± 13	25.7 ± 12.7	3.14 ± 1.21	6.56 ± 2.42	4.13 ± 1.34	1.09 ± 0.28
CONTROLS (9)	8.2 ± 3.7	24.6 ± 11.2	11.8 ± 3.6	1.99 ± 1.26	4.55 ± 2.50	2.26 ± 0.64	1.84 ± 0.3
Significance of difference (P)	< 0.01	NS	< 0.005	< 0.05	< 0.1	< 0.001	< 0.001

Table 3: Mean $\pm$ 1 S.D. of plasma insulin (mU/l), the insulin/glucose ratio (mU/l per mmol/l) and the glucose disappearance rate (KG) (% per minute) after intravenous glucose in patients and controls.

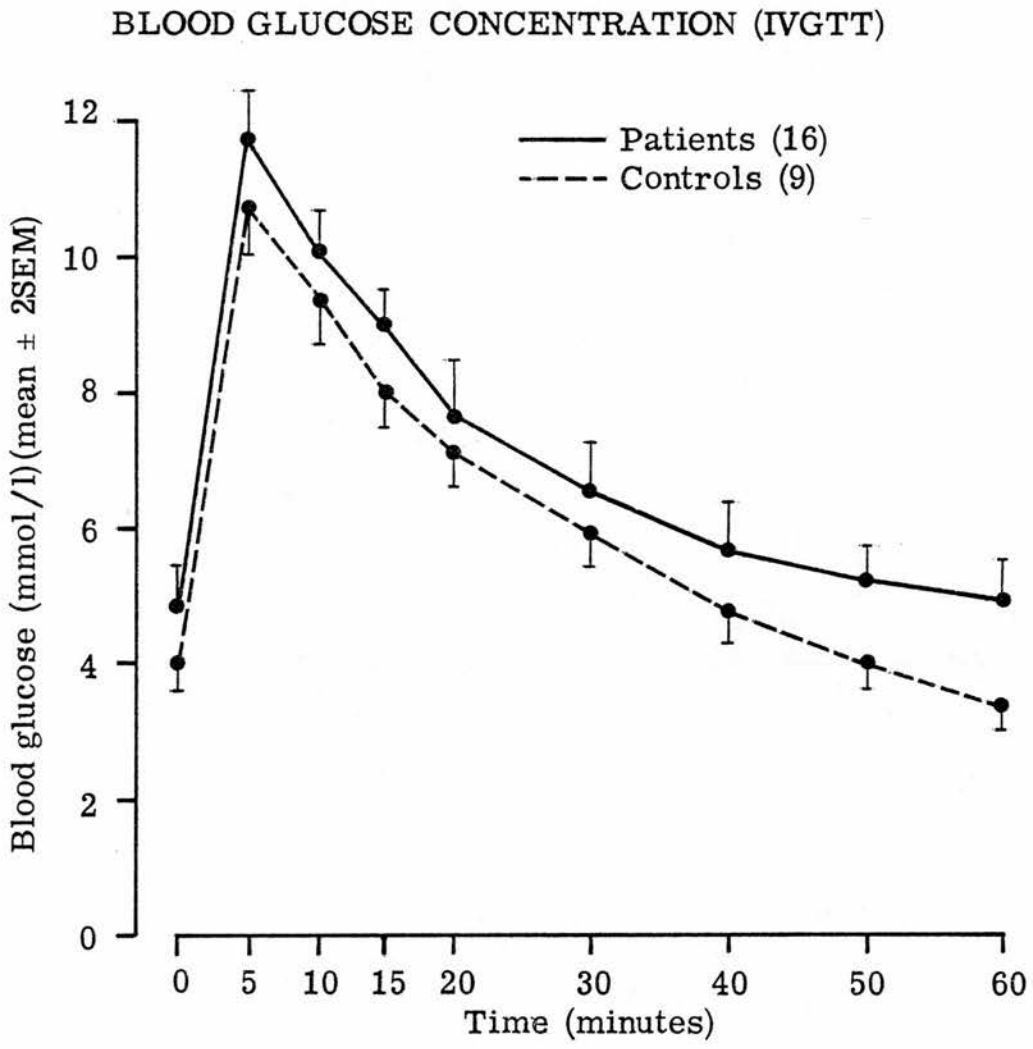

Fig. 1. Mean ± 2 SEM of blood glucose values during IVGTT in patients and controls.

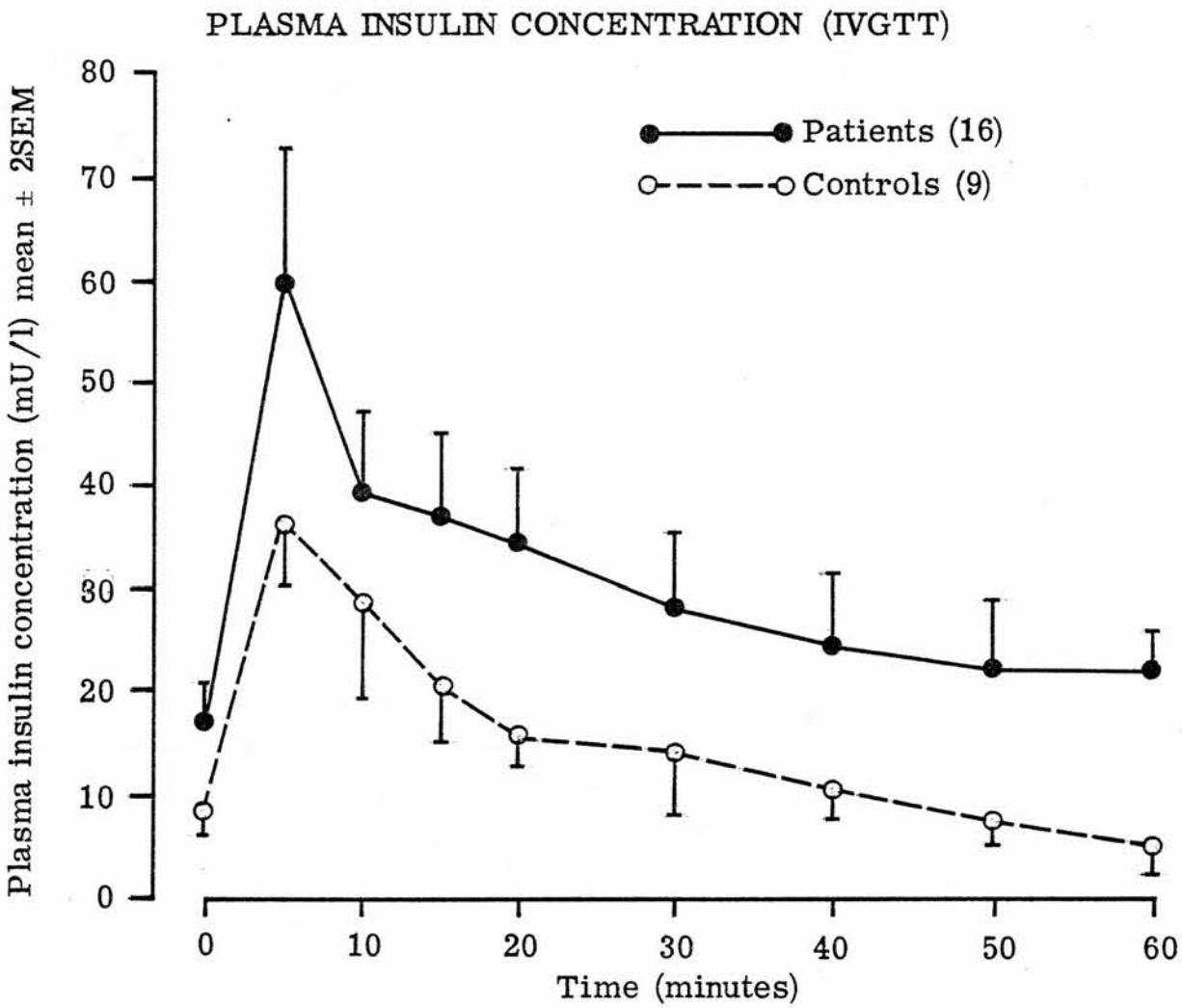

Fig. 2. Mean ± 2 SEM of plasma insulin, fasting and in response to intravenous glucose in patients and controls.

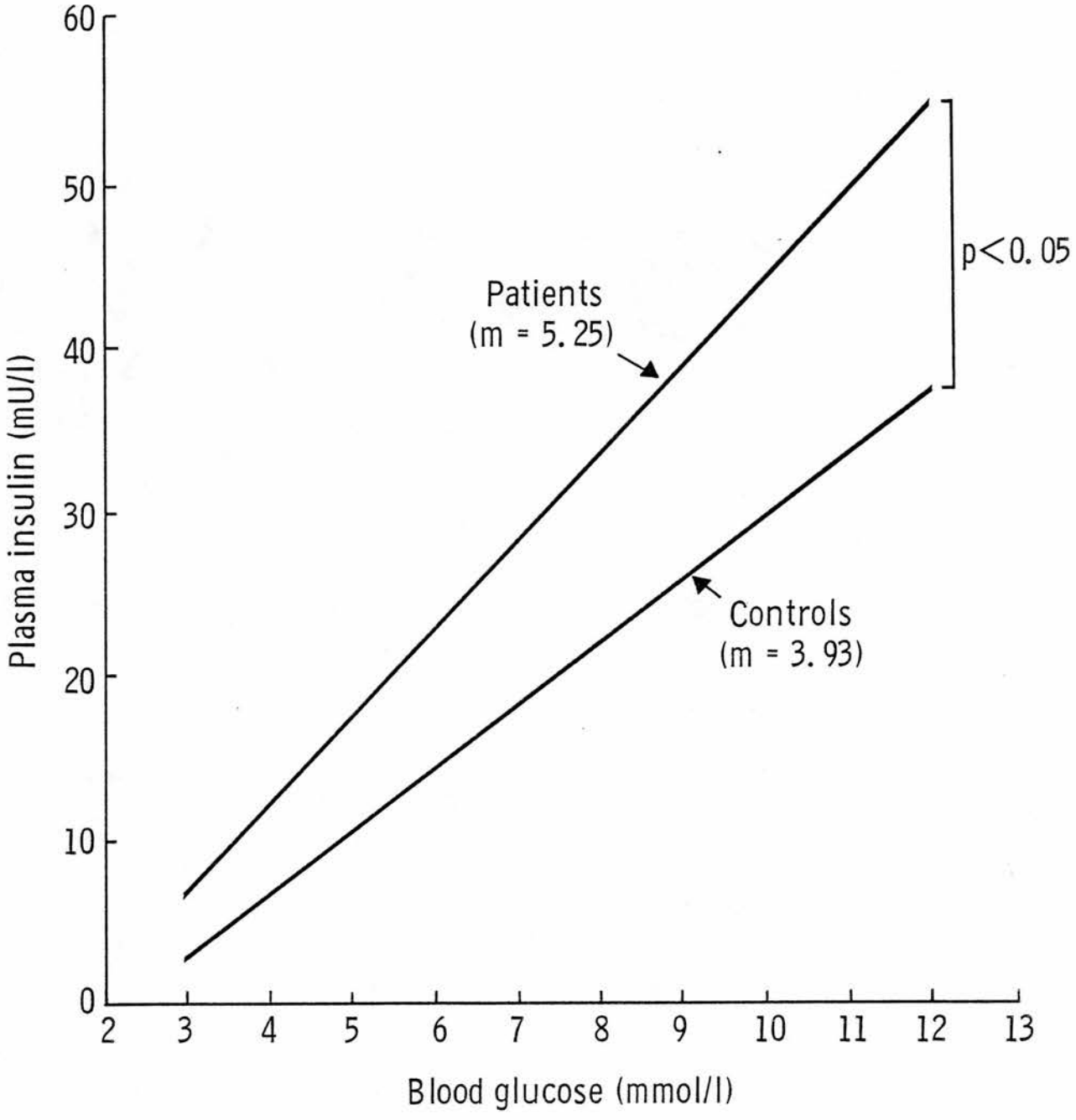

FIG.3 Plasma insulin - blood glucose relationship in patients and controls. Each line represents the mean of individual slopes for each group.

m = slope. Significance of difference (p) between mean values for patients and controls was analysed by Student's t test.

had an initial rapid increase in plasma insulin which was higher but not significantly different from that seen in the normal group. Starting from a mean fasting level of 16.7 $\pm$ 7.7 mU/L, plasma insulin increased to 60 $\pm$ 24.8 mU/L at 5 minutes. During the next 10 minutes the insulin levels fell rapidly but then declined at a much lower rate so that during the later part of the test plasma insulin levels remained significantly above normal. The peak insulin generally coincided with and correlated with peak blood glucose ($r = 0.5875$, $P < 0.02$). There was a positive relationship though not significant between K_G and the early insulin response ($r = 0.42$, $P < 0.1$). The insulin response was further analysed by examining the insulinogenic index (insulin / glucose ratio; Seltzer et al., 1967), and the regression of plasma insulin on blood glucose. The early insulin to glucose ratio was similar in patients and controls but the ratio in the latter part of the test was significantly higher in the patients than in controls (Table 3). The slope of plasma insulin - blood glucose regression was 5.25 $\pm$ 2.1 (SD) mU/L per mmol/l for the patients and 3.93 $\pm$ 1.5 mU/L per mmol/l for the controls. This slope was significantly greater in the patients than in controls (Figure 3).

Plasma growth hormone: Basal GH level and mean responses to i.v. glucose in patients and controls are shown in Table 1 and Figure 4. Fasting GH levels were significantly raised in the patients compared with controls ($P < 0.02$). In the control group there were initial increases in GH levels following glucose administration then the levels gradually declined to a nadir at 40 minutes post glucose injection with a rebound between 60 and 90 minutes. In contrast, the patients as a group exhibited a paradoxical rise in plasma GH after glucose with the maximum

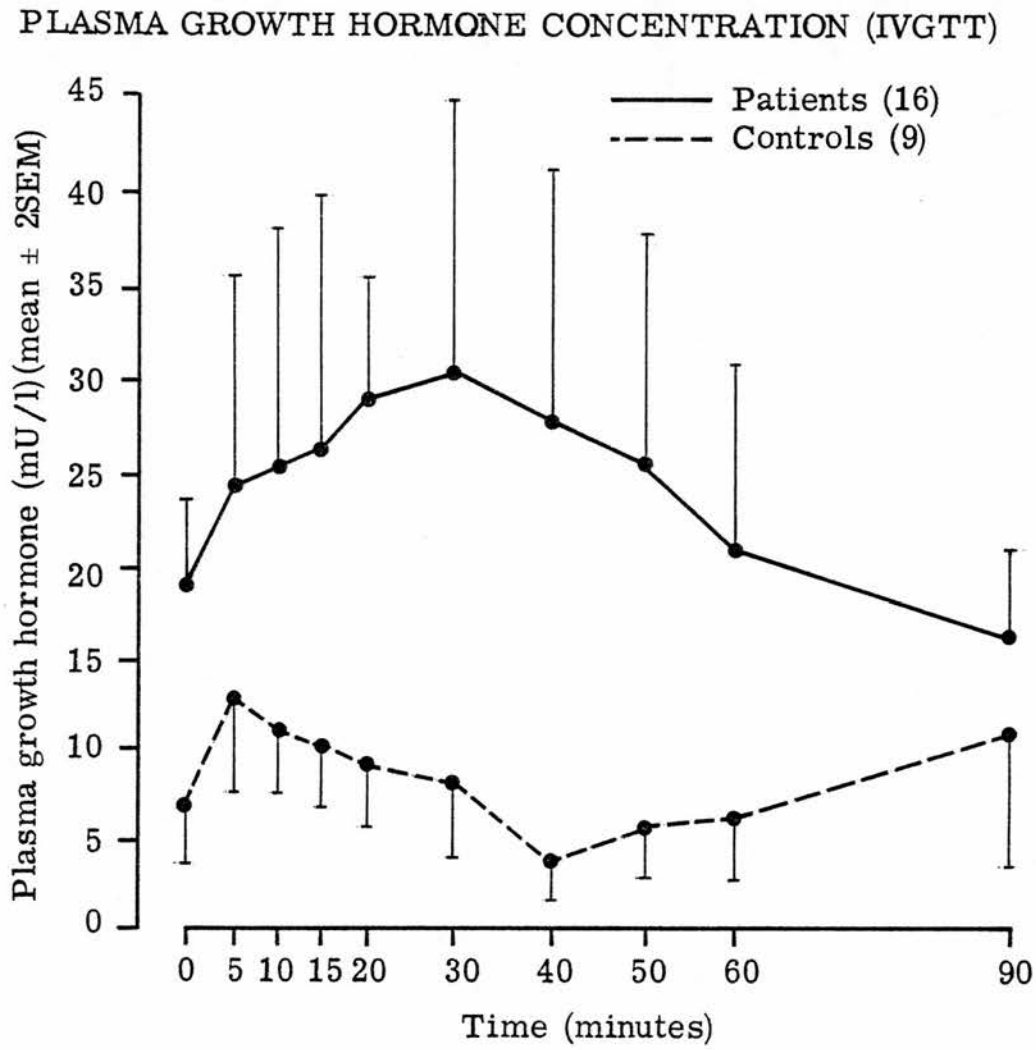

Fig. 4. Mean ± 2 SEM of plasma growth hormone, fasting and in response to intravenous glucose in patients and controls.

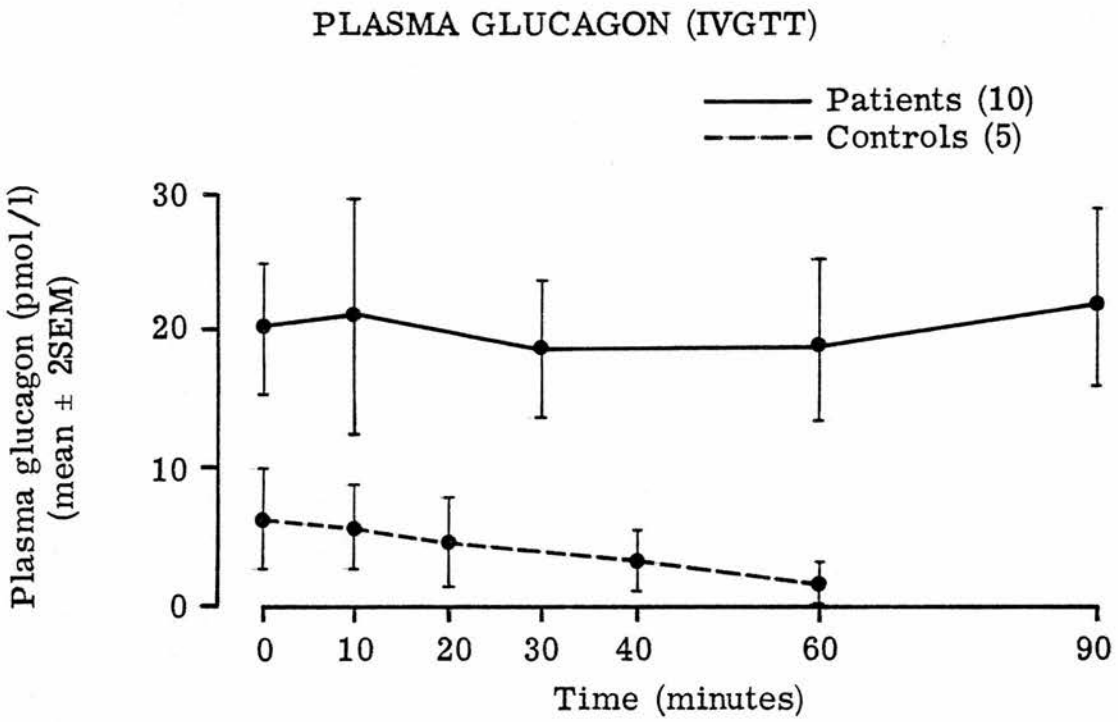

Fig. 5. Mean ± 2 SEM of plasma glucagon, fasting and in response to intravenous glucose in patients and controls.

at 30 minutes of IVGTT. There was, however, wide variation in the GH levels observed in the patients. When the growth hormone responses of the individual patients were compared with those of the normal group, the levels were suppressed by glucose in three patients, unchanged in three patients and the remainder (62.5%) showed paradoxical rises. There was no clear cut relationship between the abnormalities in GH responses and the impairment in glucose tolerance, and no correlation could be found between either fasting, peak values,or total responses of plasma GH and the degree of glucose intolerance. Plasma GH levels did not correlate with the duration of dialysis, urea or creatinine concentrations. Nor was there any relationship between diet, plasma albumin or transferrin concentrations (as indices of nutritional status) and plasma GH levels.

Plasma Glucagon: Basal plasma immunoreactive glucagon levels were significantly increased in the patients ($P < 0.005$). After glucose injection IRG fell significantly in the controls, but remained elevated throughout the test in the patients (Figure 5). The basal molar $\frac{\text{insulin}}{\text{glucagon}}$ ratio was lower in the patients than in controls but the difference was not statistically significant ($t = -1.492$, $P < 0.1$). However, the per cent increase in $M \frac{IRI}{IRG}$ at 10 and 60 minutes following glucose administration was significantly increased in the normal subjects compared with patients ($P < 0.05$ and $P < 0.02$ respectively) (Table 4). No relationship between IRG levels and the degree of glucose intolerance could be found. The elevated basal IRG did not correlate with the duration of dialysis, plasma urea, plasma creatinine, or the indices of nutritional status.

TABLE 4

	INSULIN/GLUCAGON RATIO			INSULIN/GLUCAGON RATIO (% FASTING)	
	FASTING	10 MINUTES	60 MINUTES	10 MINUTES	60 MINUTES
PATIENTS	0.29 ± 0.16	0.80 ± 0.53	0.42 ± 0.26	286.9 ± 154.8	146.5 ± 56
CONTROLS	0.49 ± 0.35	3.54 ± 4.7	1.62 ± 1.1	676.8 ± 468	445.2 ± 333.6
t	-1.492	-1.868	-3.218	-2.455	-2.857
p	NS	NS	< 0.01	< 0.05	< 0.02

Table 4: Mean $\pm$ 1 S.D. of Plasma Insulin (μU/ml)/Plasma Glucagon (pg/ml) molar ratio, fasting and in response to intravenous glucose in patients and controls.

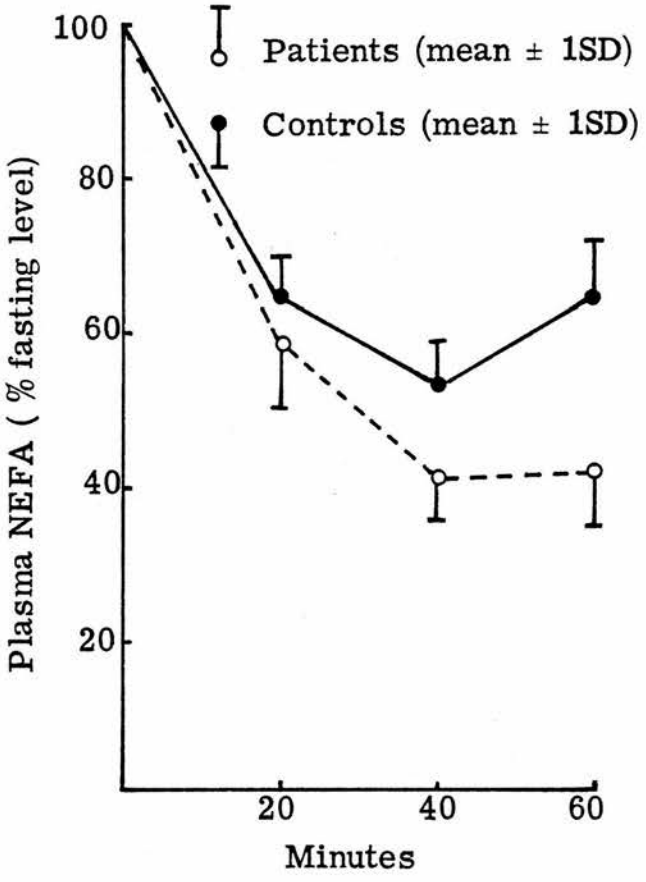

Fig. 6. Percentage fall (mean ± 1SD) of plasma nonesterified fatty acids following intravenous glucose.

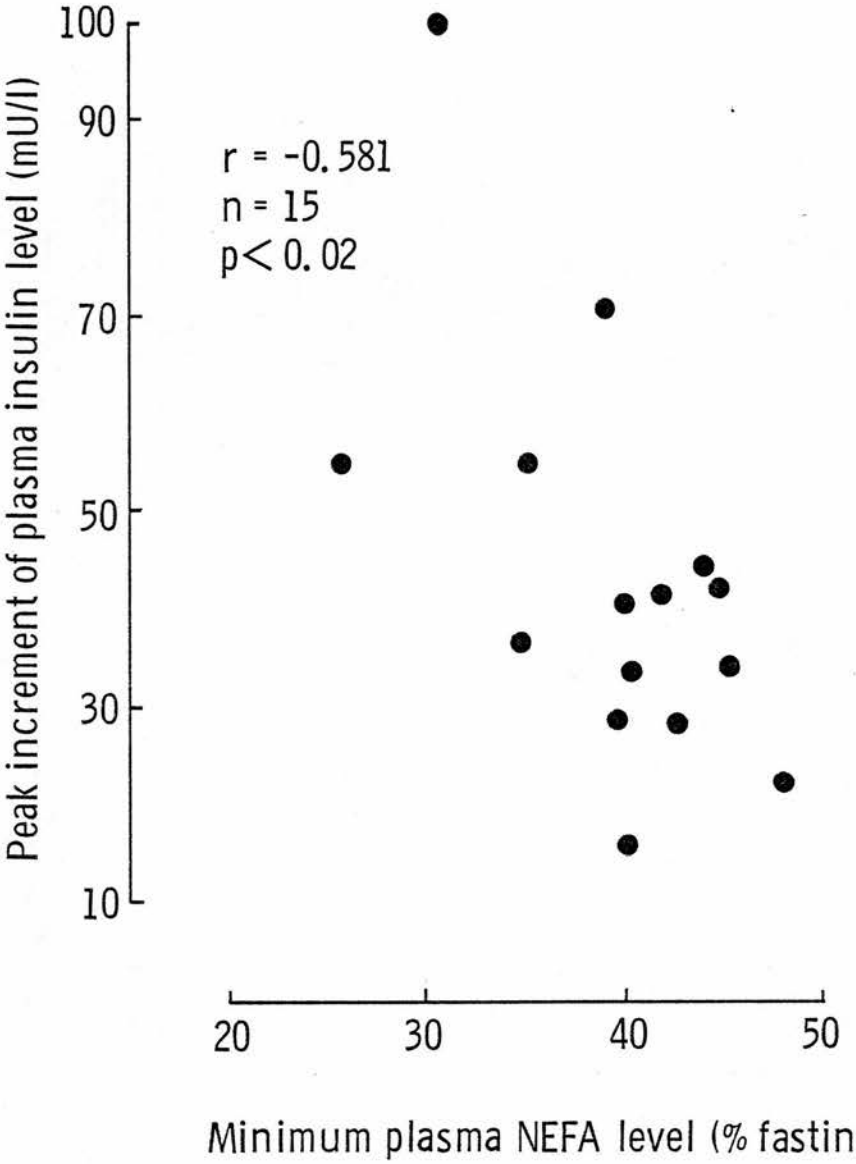

FIG. 7 Relationship between plasma insulin peak increment and maximum fall in plasma non-esterified fatty acids (NEFA) following iv. glucose

Plasma non-esterified fatty acids: The mean fasting level of NEFA in the patients was significantly lower than in controls ($P < 0.001$). Following i.v. glucose, NEFA level fell more markedly and for longer in the patients than in the controls; the percentage fall being significantly more at 40 and 60 minutes (Figure 6). There was a significant inverse relationship between the peak increment in plasma insulin level and the maximum percentage fall in plasma NEFA concentration ($r = -0.581$, $P < 0.02$ (Figure 7). No correlation was noted between fasting NEFA concentration and the degree of glucose intolerance. Basal GH levels related positively but not significantly with fasting plasma NEFA concentrations ($r = +0.44$, $P < 0.1$).

Plasma amino acids: The means and standard deviations of plasma amino acid concentrations in the fasting state and 60 minutes after intravenous glucose in the patients and controls are shown in Table 2. Significant decreases in fasting plasma amino acid concentrations, compared with controls, were found in valine, leucine,isoleucine, lysine, histidine, tyrosine and serine; and increases in glycine, and citrulline. There was a significant mean reduction in the concentration of valine, leucine and isoleucine, tyrosine, alanine, lysine and serine 60 minutes after glucose injection. Other amino acids also fell in concentration, but not significantly. These changes did not correlate with the peak, or the 60 minutes plasma insulin concentrations. The changes in plasma amino acid concentration in the controls are also shown in Table 2, and there were significant reductions in valine,leucine, isoleucine, glycine, alanine and tyrosine.

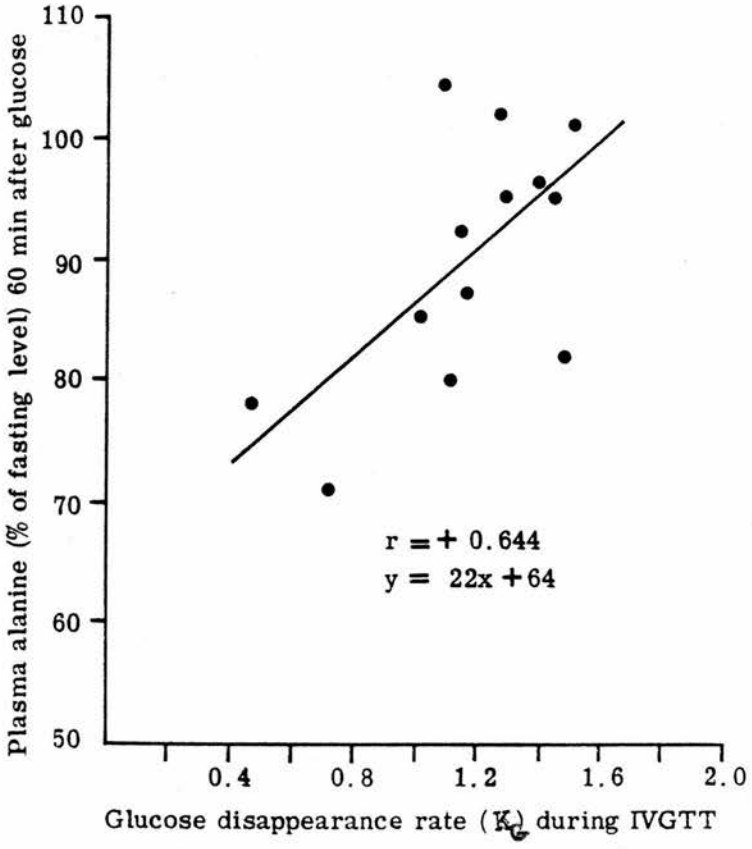

Fig. 8. Relationship between the percentage fall in plasma alanine concentration 60 minutes after glucose injection and the K values for glucose during IVGTT in patients.

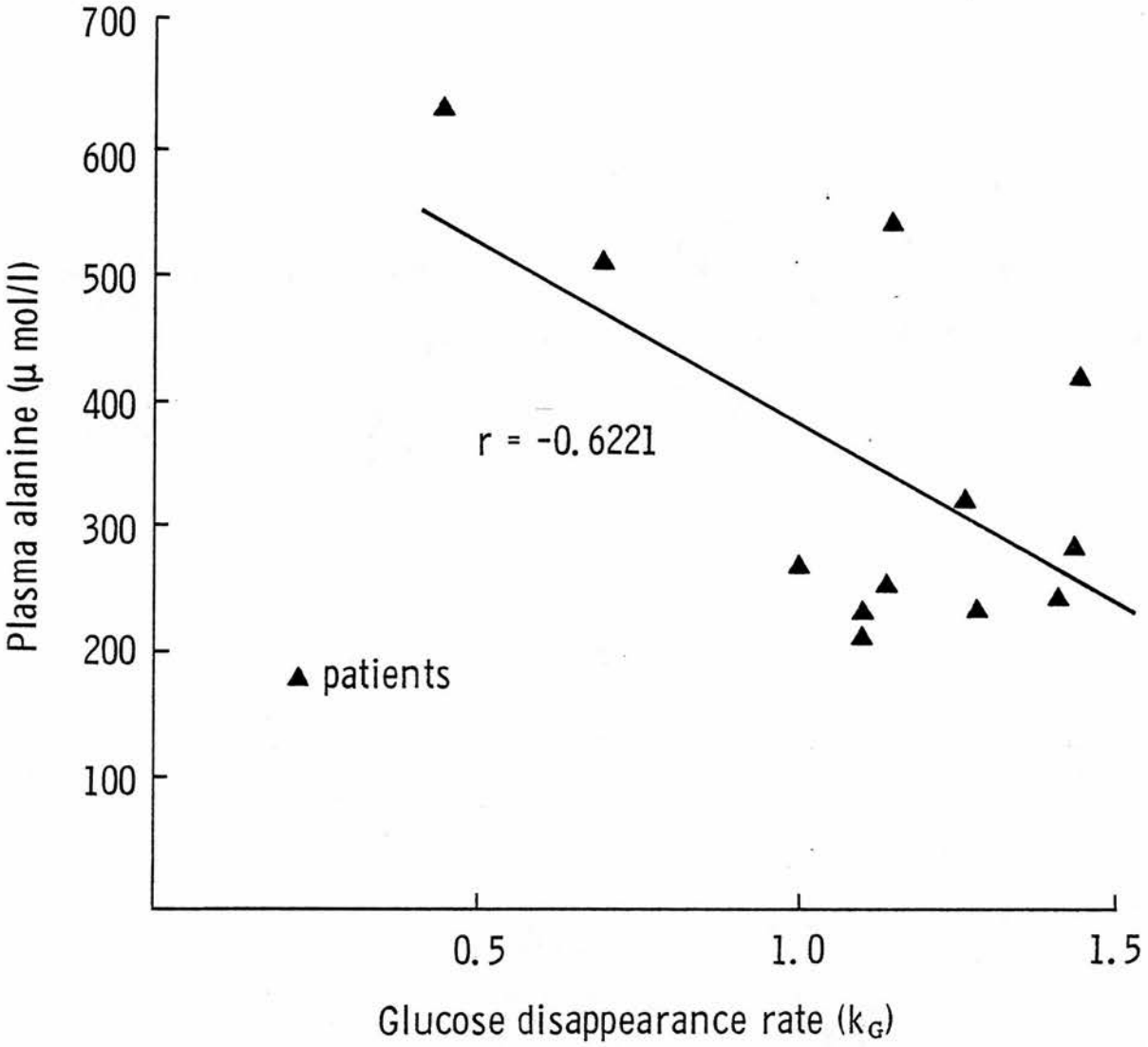

FIG. 9 Relationship between glucose disappearance rate and fasting plasma alanine concentrations.

The concentration of alanine 60 minutes after glucose injection was expressed as a percentage of the fasting level and compared to the glucose disappearance rate (K_G) during the IVGTT. There was a close linear relationship between the percentage reduction and K_G values (Figure 8). The fasting concentrations of alanine correlated inversely with K_G values ($r = -0.62$; $P < 0.05$) (Figure 9); thus, the worse the glucose tolerance, the higher the fasting concentration of alanine and the greater its fall after glucose. Those with better glucose tolerance had normal plasma alanine concentrations, which showed little or no change following i.v. glucose. These findings did not apply to any of the other amino acids.

DISCUSSION

This study confirms the findings of others that impaired carbohydrate tolerance is very common in patients with chronic renal failure and extends it to children on haemodialysis. Blood glucose was measured by an automated ferricyanide reduction method (Auto-analyser ,Technicon), which is not specific for glucose and certain non-glucose reducing substances are also measured. Powell and Djula (1971) have shown this method to result in higher values in uraemia compared with glucose-oxidase method. However, the interfering substances do not change during the glucose tolerance test and therefore the dynamic changes of blood glucose during the test are valid (Briggs et al., 1967; Richards et al., 1977).

The data clearly indicate that the patient's ability to secrete insulin, particularly in the first few minutes following an intravenous glucose load, is normal. This excludes a defect in pancreatic insulin secretion as a cause of the glucose intolerance. The observation of others (Garcia et al., 1966) that it is the magnitude of early secreted insulin in IVGTT that is best correlated with the rate of glucose utilization, supports this conclusion. Furthermore, as an index of the appropriateness of any given insulin response, the early insulin to glucose ratio was found to be normal in this study. This is in accordance with the findings of most investigators (Briggs et al., 1967; Lowrie et al., 1970; Spitz et al., 1970). Plasma insulin level is a function of both insulin secretion and degradation rate. During thd latter part of the test the patients maintained plasma insulin levels and insulin/glucose ratio that were significantly greater than normal. Although this elevation could be due in part to a decreased insulin clearance rate

(a well established fact in uraemia), continued stimulation of insulin secretion could also have occurred. The present study was not designed to separate these two functions. A more important factor in the development of an abnormal carbohydrate tolerance in uraemia appears to be the peripheral resistance to insulin action particularly in muscle (DeFronzo, 1978). In the present study this was manifested by the raised blood glucose concentrations inspite of elevated plasma insulin levels, the presence of high insulin/glucose ratio and the greater slope of the insulin-glucose regression in the patients compared with controls. Thus, each increment in blood glucose was correlated with a greater increment in plasma insulin. The degree of glucose intolerance did not correlate with plasma GH, IRG or NEFA and the precise nature of this resistance remains obscure.

It is of interest that fasting plasma NEFA levels were significantly lower in the patients and fell more markedly following glucose administration. This suggests a decrease in lipolysis and/or increased re-esterification of NEFA in the uraemic state due to high insulin levels, and that there seems to be no decrease in sensitivity of adipose tissue to the antilipolytic action of insulin. This relative sensitivity of adipose tissue has been shown to occur in catabolic situations characterised by carbohydrate intolerance such as peritonitis (Ryan et al., 1974) and following thermal injury (Batstone et al., 1976). Some indication of a decrease in NEFA mobilisation from adipose tissue in the uraemic state was also shown in studies by Nitzan et al. (1968); when rats were made uraemic and then subjected to two days fasting period they exhibited hyperglycaemia and hyperinsulinaemia and had levels of

NEFA that were significantly lower than those measured in sham-operated controls submitted to an equal fasting period. Similar metabolic state was also described in starved-septic rats (Blackburn and Flatt, 1974). Because the uraemic patient may have a decreased ability to mobilize endogenous fat for energy production in the face of a decrease in glucose utilization, the supply of metabolic fuels to tissues such as muscle is reduced, except for amino acids the intracellular pool of which is continuously fed by protein breakdown. This will result in an increase in protein degradation to supply the substrate. It is known that certain amino acids particularly the branched-chain amino acids can be rapidly oxidised in muscle (Manchester, 1965), and if their catabolism is increased a concomitant increase in the formation of alanine will ensue (Garber et al,1976).Thus disproportionately high levels of alanine are found in the plasma under conditions of protein wasting (Felig et al., 1970; Adibi, 1976). The basal reduction of the plasma branched-chain amino acids and the elevation of alanine observed in the patients of this study might therefore be an indication of increased oxidation of essential amino acids with increased alanine formation and peripheral release. This is in keeping with the correlation found between fasting plasma alanine levels and the degree of glucose intolerance.

The fall in plasma amino acid concentrations after glucose in the patients and controls was similar to that described in healthy adults (Crofford et al., 1964). It is considered to be insulin-mediated as it does not occur with insulin deficiency (Diabetes) (Zinneman et al., 1966). The results have demonstrated that the metabolic reactions initiated by a glucose load, which leads to the removal of essential

amino acids from plasma under the influence of insulin is operative in children on haemodialysis for chronic renal failure. If the disappearance of plasma amino acids is directly linked with the synthesis of protein by muscle, as implied by many authors, (Swendseid et al., 1967), it would mean that the mechanisms for protein synthesis are maintained, though not necessarily normally, in the muscle of uraemic children. The fall in the concentration of alanine (from high to normal levels) is of interest. In normal subjects, no change or even an increase after glucose or insulin has been reported (Crofford et al., 1964; Felig et al., 1969). This could not be related to the key role of this amino acid in gluconeogenesis where a higher level would have been expected from a "shutting off" of this mechanism after glucose load. The fall in circulating alanine levels suggested that alanine formation and/or release in the periphery had been influenced by glucose administration. Similar fall in plasma alanine concentration has been described in malnourished children in response to oral glucose load, but this effect did not occur after recovery (Grimble and Whitehead, 1971).

Basal hyperglucagonaemia is well recognised in uraemic adults and is due probably to increased secretion as well as decreased degradation of plasma glucagon (Bilbrey et al., 1974; Sherwin et al., 1976). Although noh-suppressable in this study, Kuku et al. (1976), in demonstrating that it is a heterogeneous molecule, showed that the fragment which corresponds to the biologically active glucagon did fall in concentration after glucose. The assay for glucagon used in the present study was not specific for the active component and the persistently

elevated levels during IVGTT might be due to non suppressibility of the other fragment. It is not known why glucagon secretion is increased in chronic renal failure. It could be a consequence of continuous supply of glucagon-stimulating amino acids entering the circulation, mainly alanine, a known potent stimulant of glucagon secretion, or it may be that glucagon, in response to a relative "glucose - lack" induced by failure of normal glucose transport and metabolism, is secreted in an attempt to correct that "lack" via hepatic gluconeogenesis as in diabetes mellitus (Unger et al., 1970), or due to direct stimulation by retained nitrogenous compounds mainly guanidines (Cohen, 1976). Whatever the cause, the uraemic state is characterized by markedly elevated basal glucagon levels and hyposuppressibility of the pancreatic alpha cells by glucose, resistance to insulin, and hypersensitivity to glucagon (Sherwin et al., 1976).

According to the concept that glucagon is a hormone of catabolism and that on molar basis it is more powerful than insulin with respect to its action on hepatic glucose balance (Unger, 1971), therefore, the inability of the patients in this study to increase the insulin to glucagon molar ratio in a normal manner following exogenous glucose (assuming that the measured plasma IRI and IRG were biologically active) is indicative of reduced anabolism despite the supply of exogenous source of energy. This may help explain the observation made in Study I why some of these children failed to sustain growth in spite of seemingly adequate caloric intake.

Alanine, the major gluconeogenic substrate, was found to be high in those patients with poor glucose tolerance. Since substrate availability

is the main drive for hepatic gluconeogenesis (Felig et al., 1969; Exton, 1972), and in keeping with hyperglucagonaemia, gluconeogenesis might be increased in these children and could have contributed to the glucose intolerance. Recent studies by Garber and his colleagues (1978) support this notion. They demonstrated increased alanine production and release from skeletal muscle of chronically uraemic rats. However, the negative correlation between plasma alanine and K_G found in the present study may not be a cause and effect relationship but a reflection of abnormalities of both variables associated with the severity of the disease.

In prolonged starvation hypoalaninaemia is the rule with a decrease in gluconeogenesis as a result of increasing utilization of endogenous fat for energy and, therefore, sparing body protein . Such a metabolic adaptation process may be denied to the uraemic patient because of reduced availability of endogenous fat and perhaps even a defect in its oxidation (Williams and Luft, 1976, abstract). It follows that the catabolic response to fasting will be more profound in uraemia. This has been shown to be true in the uraemic rat model (Holliday et al., 1977).

As previously discussed, the elevated plasma GH levels are due to increased secretion rather than impaired plasma clearance. It is possible that the intracellular energy supply is deranged in uraemia and that GH secretion is increased in an attempt to mobilize fat for energy production and preserve body protein. The rise in GH levels after glucose is interesting and a similar phenomenon has been described in a variety of catabolic illnesses. Although a clear explanation of

this phenomenon does not exist, various suggestions have been made ; (1) The stimulatory effect of chronic protein depletion upon GH secretion overrides the inhibitory effect of the induced hyperglycaemia (Pimstone et al., 1967), and (2) An abnormality exists in hypothalamic function (Alvarez et al., 1972). It is possible, however, that the normal GH response to glucose is ablated in uraemia because the marked fall in NEFA levels, due to hyperinsulinaemia, stimulates its secretion. There is evidence that plasma NEFA have a regulatory effect on GH secretion (Blackard, 1973).

The use of intravenous glucose as a tool to investigate hormonal and metabolic interrelationships has revealed different responses in children with chronic renal failure, compared with healthy children. On the basis of these findings I speculate that the prime defect is a failure of normal glucose uptake by cells and decreased fat availability for energy production leading to increased protein degradation and in the case of children failure of growth. More detailed metabolic studies , perhaps in animal model, are required to elicit further the hormonal and fuel homeostasis in uraemia. This will be of great help in the practical treatment of these children particularly in the strategy for dietary therapy.

CHAPTER 9

STUDY III.

OXYGEN CONSUMPTION AND BODY COMPOSITION.

Energy equilibrium in a normal child is the balance between energy intake and energy expenditure. The latter relates to physical activity, basal metabolism and growth.

The observation made in Study I that statural growth of uraemic children remained poor despite apparently adequate energy intake suggest that chronic renal failure per se may increase maintenance energy requirement above normal, either by decreasing the efficiency of energy utilization at a cellular level or by increasing basal energy expenditure or both.

Recent investigations have shown a close relationship between basal energy expenditure and protein turnover particularly in individuals recovering from catabolic conditions (Kien et al., 1978) and children recovering from protein-energy malnutrition (Brooke and Ashworth, 1972). Knowledge of energy requirements for basal metabolism in children with chronic renal failure will, therefore, be important in order to estimate the energy intake required to maintain energy balance and net protein anabolism. This study evaluated basal energy expenditure in this group of children on haemodialysis by measuring basal oxygen consumption.

Adult patients with chronic renal failure have an abnormally high content of body water and most of this excess is believed to be in the extracellular phase (Coles, 1972; Comty, 1968). As basis of

of reference, therefore, the body cell mass is preferable to the body weight. For this reason and to evaluate the effects of chronic renal failure on body composition in children, concomitant studies were made of total body water (TBW) and extracellular water (ECW), and the oxygen consumption was considered in terms of the metabolically active body cell mass (BCM).

MATERIALS AND METHODS

Patients and Controls

Body composition and basal oxygen consumption studies were carried out during the period of growth observation referred to in Study I. Body composition was determined in 13 of the 16 patients. All were clinically stable and none was oedematous or hypertensive at the time of the investigations. The mean interval between the last dialysis session and commencement of the study was 14.8 hours. Patients number 1, 7 and 8 refused the investigations. For ethical reasons body composition studies could not be carried out in normal children and the patients acted as their own controls (see Methods).

With the exception of patient number 15 who was not available for the study, basal oxygen consumption was determined in all cases approximately within two weeks of the body composition study. As the patients were growth retarded, control values were obtained from ten healthy children matched for height. Four of the controls were children with familial short stature but otherwise entirely normal. The remainder were normal healthy children. Clinical data of the control group are shown in Table I.

Clinical data and management of the patients have already been described (Chapter 7).

TABLE I

No.	Sex	Age (yrs)	Weight (kg)	Height (cm)
1	M	12	27.5	133
2	M	12	30	139
3	M	11	20	110
4	F	12.5	27	135
5	M	13.5	34	138
6	F	13.6	48	165
7	F	11	45	140
8	F	13.4	51	138
9	F	11.5	48	146.5
10	M	14	55	163

Table 1: Clinical data of control subjects.

METHODS

Body composition

Height and weight were recorded at the commencement of the study. Triceps and subscapular skinfold thickness were measured using a Harpenden skinfold caliper, according to the method of Tanner and Whitehouse (1975).

Total body water (TBW) was measured using tritiated water dilution techniques. A dose of 3 m cu/kg body weight tritiated water was administered intravenously by a syringe weighed before and after the injection. Blood samples were taken hourly from two to six hours, during which nothing was permitted by mouth. As the patients were anuric, no allowance was made for urinary loss. Plasma water was extracted from plasma by vacuum distillation under acetone and dry ice (Moss, 1964), and counted in an LKB- Walloc 81000 liquid scintillation counter. The tritium activity was assayed with the use of a liquid scintillation system (Langham et al., 1956) and allowance was made for quenching by internal standards. Counting efficiency was approximately 25%. TBW was calculated using the usual isotope dilution formula (Forbes, 1962). Equilibrium appeared complete by three hours : the two hour and three hour samples were significantly different (paired 't' test, $p < 0.02$), whereas no difference was detected between the three and six hour plasma samples. TBW was, therefore, taken as the mean of the three to six hour samples.

Extracellular water (ECW) was estimated from the bromide distribution space using a fluorescence excitation of non-radioactive bromine method (Kaufman and Wilson, 1973). Non-radioactive sodium bromide, 3% solution at a dose of 0.4 meq/kg body weight, was given intravenously immediately after the tritiated water and blood samples taken at four and six hours. Plasma bromide was measured by fluorescence excitation in a nuclear data 100 multichannel analyser, using a cadmium source (Kaufman and Wilson, 1973). As there was no significant difference between the four and six hour samples, ECW was estimated from the four hour sample.

Derived Data

(i) Intracellular water (ICW) was calculated by subtracting ECW from TBW (TBW-ECW).

(ii) Total body solids were calculated from the formula :

$$TBS\ (kg) = BW\ (kg) - TBW\ (1)$$

where BW = body weight.

(iii) Total body fat (TBF) was calculated from the nomogram for the variable hydration of the fat-free body (FFB) of Moore et al. (1963) using the ratios ICW/TBW and ECW_o/ECW_p, where ICW-intracellular water, TBW-total body water, ECW_o - observed extracellular water, ECW_p - predicted extracellular water:

$$TBF = \text{body weight} - FFB$$

(iv) Fat-free solids (FFS) were calculated from the formula :

$$FFS\ (kg) = FFB\ (kg) - TBW\ (L)$$

(v) Body cell mass (BCM) was estimated from a linear regression equation relating it to intracellular water (ICW). The equation was calculated from the reported regression of ICW on whole body exchangeable potassium (ke) and a direct proportionality constant relating Ke and body cell mass (Moore et al., 1963):

$$BCM = 9.1\ Ke \text{ and } Ke = 0.149\ ICW + 0.061$$

$$\text{thus, } BCM = 1.356\ ICW + 0.5551.$$

Predicted Data

Normal body composition was predicted for each patient from the observed body weight and height at the time of assessment, and the

results obtained were expressed as a ratio of observed/predicted:

(i) TBW - predicted value for TBW was obtained from the following multiple regression equations on both height and weight for normal boys and girls (Cheek,1968):

Boys -

(a) $Ht \geq 137.22$ cm :

$$TBW = 0.381\ (wt) + 0.257\ (Ht) - 28.985$$

(b) $Ht \leq 137.22$ cm :

$$TBW = 0.397\ (wt) + 0.067\ (Ht) - 2.778$$

Girls -

$Ht \geq 113.02$ cm :

$$TBW = 0.320\ (wt) + 0.135\ (Ht) + 9.577$$

(ii) Cheek (1978) has shown that intracellular water (ICW) has a strong linear relationship to the cube of the height in both prepubertal children and pubertal children with little variation in males and females. Therefore, ICW was predicted from the following regression equation relating it to the cube of the height (Cheek, 1978):

$$ICW = 3.782\ (Ht)^3 + 1.753$$

(iii) Extracellular water was predicted from the formula :

$$ECW = TBW - ICW.$$

(iv) Fat-free body (FFB) was predicted from the formula :

$$FFB = \frac{TBW}{0.73}$$

assuming that, in normal individuals, FFB has 73% water content.

(v) Predicted values for TBS, FFS and TBF were obtained as described for derived data above.

Oxygen Consumption Measurement

Oxygen consumption measurements were made with a 'flow through' gas analyser, Noyons Diaferometer (MG4 Universal Diaferometer,Kipp and Zonen, Deft, Holland)(Fig.1). This utilizes an open system in which the subject breathes, under translucent plastic hood over the head and neck, room air that is suctioned through at a rate appropriate for size. The instrument analyzes difference in the oxygen and carbon dioxide concentration of inspired and expired air by measuring differences in heat conductivity. The principle of the method is fully described by Kleiber (1961). The diaferometer was calibrated at regular intervals with O_2 and CO_2 at known flow rates.

Procedure

The morning after an overnight fast of not less than 12 hours the children were rested for at least one hour prior to the commencement of the study. The child,dressed in light clothing, lies with the head and the neck under a plastic hood. A suction pump draws room air into the hood around a loose neck seal, over the subject's face, and through a corrugated tube into the gas analysing system. Air-flow rate of 50 l/min and hood size appropriate for children were used. After approximately 15 minutes, allowing the child to get used to the test, measurements were carried out according to the following procedure :

1 - Room temperature and barometer pressure were read.

2 - The instrument was set on "base line" and room air was analyzed continuously for O_2 and CO_2 with readings

FIG.1. APPARATUS USED FOR THE MEASUREMENT OF OXYGEN CONSUMPTION

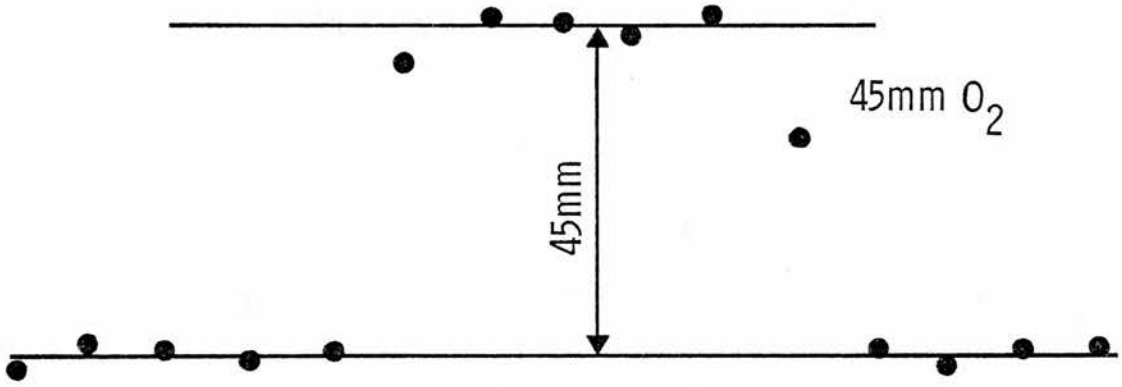

FIG. 2 Typical graph obtained for base and deflection lines for Oxygen uptake measurement.

taken every minute for five minutes after the galvanometers have reached a stable position. The readings were plotted on the mm - grid of graphical paper, the horizontal axis being used as time axis (e.g. 1 cm for 1 minute).

3 - The instrument was then set on "deflection" for analysis of the mixture of expired air and room temperature, and the positions of the galvanometers were taken again every minute until the deflections were constant. These readings were also plotted.

4 - Subsequently, the instrument was set back to "base line" and the position of the galvanometer was read every minute until the deflection was again constant. The base line and the deflection line are parallel, and the two parts of the base line obtained before and after the deflection line form a straight line. The vertical distance between the two lines gives the value of the deflection in mm (Fig. 2).

Two measurements were made for each child and the average taken. The percentage differences between the two values ranged from 2 - 8%. None of the children were restless and most of them fell asleep during the test.

Calculations of Results

The results were obtained in terms of mm deflection for oxygen. The temperature during the measurements was indicated by a thermometer on the instrument and the corresponding calibration constants, listed by the manufacturers, were used in the evaluation of results.

The concentration difference for oxygen in the sample stream with respect to the reference air was calculated by multiplying the measured deflections with the calibration constant:

1 - percentage oxygen decrease ($\triangle O_2$):

$$\triangle O_2 = \text{constant } O_2 \times \text{deflection } O_2.$$

The oxygen uptake of the subject was evaluated from the measured oxygen percentage and the flow rate used in the measurement.

2 - Oxygen uptake :

$$\text{litres } O_2 / \text{min} = \text{flow rate (1/min)} \times \triangle O_2/100 \times \mu.$$

By means of the correction factor μ the measured litres were converted to normal litres (at 0° centigrade and 760 mm Hg barometric pressure). A nomogram for the evaluation of μ from room temperature and barometric pressure was supplied with the diaferometer.

The values for oxygen uptake were expressed in terms of body surface area, height and body cell mass for both patients and controls.

For ethical reasons, body composition studies on control children were not carried out. Intracellular water (ICW) was therefore estimated from a linear regression equation relating it to the cube of the height (Cheek, 1978) and body cell mass was then predicted from ICW as described above.

RESULTS

(A) BODY COMPOSITION

Anthropometric measurements of the patients at time of study are shown in Table 2.

The observed, derived and predicted values of body composition determinants in the patients are shown in Table 3. The relationships between observed body composition and predicted normal values for the same weight and height are presented in Table 4.

Total Body Water (TBW)

Total body water ranged from 54.2 to 73.4% of body weight with a mean of 63.03%. The TBW ratio was increased in ten patients (1.01 to 1.19) and was reduced in three patients (0.92, 0.98 and 0.98), as seen in Table 4. Variations in TBW ratios did not parallel ECW ratio deviations, and were always less marked. The difference between the observed and the predicted TBW was of a borderline significance ($p < 0.05$).

Extracellular Water (ECW)

Extracellular water ranged from 23.6 to 37.1 per cent of body weight (mean 30.6%). ECW ratio was increased in variable degree in all patients ranging from 1.11 to 1.62 (mean 1.27).

Intracellular Water (ICW)

As shown in Table 3, ICW ranged from 27.3 to 42 per cent of body weight (mean 32.5%). ICW ratio was reduced in eight of the patients

TABLE 2

Case No.	Sex	Height	Weight	Puberty Status	Skinfold thickness (mm)	
					Triceps	Subscapular
2	M	144.1	42.0	PUB	18.0	16.0
3	F	154.4	48.0	PUB	27.6	20.1
4	M	162.5	45.7	PUB	4.5	6.1
5	M	142.5	29.5	PRE	14.5	4.2
6	F	143.0	31.2	PRE	12.5	5.3
9	F	129.1	22.5	PRE	7.5	5.7
10	M	142.1	55.5	PUB	7.6	7.7
11	M	149.0	38.7	PUB	13.4	6.3
12	F	128.7	25.9	PRE	7.1	5.1
13	M	130.5	31.4	PRE	15.3	13.9
14	M	131.1	31.5	PUB	11.2	10.7
15	F	140.6	51.0	PUB	21.3	24.9
16	F	141.3	41.5	PUB	14.1	15.6

Table 2: Clinical data of patients at the time of body composition study.

TABLE 3

Case No.	TBW		ECW		ICW		BCM	
	litres	%BW	litres	%BW	litres	%BW	kg	%BW
2	25.13	59.8	11.30	26.9	13.83	32.9	19.3	45.9
3	26.85	55.9	13.18	27.5	13.67	28.5	19.1	39.8
4	33.56	73.4	14.35	31.4	19.21	42.0	26.6	58.1
5	19.64	66.6	10.05	34.1	9.59	32.5	13.5	45.7
6	19.33	61.9	10.80	34.6	8.53	27.3	12.1	38.8
9	15.52	68.9	8.34	37.1	7.18	31.9	10.3	45.8
10	22.62	63.7	10.81	30.4	11.81	33.3	16.5	46.5
11	23.62	60.9	12.53	32.3	11.28	29.1	15.8	40.8
12	18.78	72.5	9.14	35.3	9.64	37.2	13.6	52.5
13	17.02	54.2	8.89	28.3	8.13	25.9	11.5	36.6
14	20.02	63.6	9.03	28.7	10.99	34.9	15.4	48.9
15	30.75	60.3	12.08	23.6	18.67	36.6	25.8	50.6
16	23.90	57.6	11.48	27.7	12.42	29.9	17.4	55.2
Mean	22.82	63.03	10.9	30.9	11.90	32.5	16.68	46.5
S.D.	5.28	5.9	1.8	3.6	3.73	4.5	5.04	6.5

Table 3: Results of body composition in absolute values and as percentage of body weight.

TBW - total body water; ECW - extracellular water;
ICW - intracellular water; BCM - body cell mass;
TBS - total body solids; FFS - fat-free solids;
TBF - total body fat; BW - body weight.

TABLE 3 (continued)

Case No.	TBS		FFS		TBF	
	kg	%BW	kg	%BW	kg	%BW
2	16.9	40.2	9.3	22.1	7.6	18
3	21.2	44.1	8.5	17.7	12.7	26.4
4	12.2	25.7	10.0	21.9	2.6	4.7
5	9.8	33.4	5.6	18.9	4.3	14.5
6	11.8	38.1	5.2	16.6	6.7	21.5
9	6.9	31.1	4.1	18.2	2.9	12.9
10	12.9	36.3	6.4	18.0	6.5	18.3
11	15.1	39.1	7.1	18.3	8.1	20.8
12	7.1	27.5	5.0	19.3	2.1	8.1
13	14.4	45.8	5.7	18.1	8.7	27.7
14	11.5	36.4	6.7	21.3	4.8	15.6
15	20.2	39.7	11.6	22.7	8.9	17.5
16	17.6	42.4	8.0	19.3	9.6	23.1
Mean	13.66	36.9	7.1	19.4	6.5	17.8
S.D.	4.49	6.1	2.1	1.9	3.1	6.6

TABLE 4

Case No.	$\frac{TBW(0)}{TBW(P)}$	$\frac{ECW(0)}{ECW(P)}$	$\frac{ICW(0)}{ICW(P)}$	$\frac{BCM(0)}{BCM(P)}$	$\frac{FFS(0)}{FFS(P)}$	$\frac{TBF(0)}{TBF(P)}$	$\frac{TBS(0)}{TBS(P)}$
2	1.04	1.03	1.05	1.05	1.04	0.83	1.05
3	1.01	1.20	0.87	0.87	0.86	1.1	0.87
4	1.17	1.17	1.07	1.07	0.89	0.49	1.07
5	1.04	1.62	0.75	0.76	0.79	1.19	0.76
6	0.98	1.54	0.66	0.67	0.71	1.59	0.67
9	1.03	1.62	0.72	0.74	0.73	1.52	0.74
10	1.07	1.28	0.94	0.94	0.82	0.98	0.94
11	0.98	1.27	0.79	0.79	0.80	1.37	0.79
12	1.17	1.46	0.98	0.98	0.84	0.54	0.98
13	0.92	1.07	0.80	0.80	0.84	1.40	0.80
14	1.08	1.11	1.05	1.05	0.97	0.79	1.05
15	1.19	0.89	1.52	1.50	1.22	0.56	1.50
16	1.05	1.11	1.00	1.00	0.95	0.93	1.00
AVERAGE	1.04	1.27	0.90	0.94	0.86	1.09	0.94

Table 4: Relationship between observed (0) and predicted (P) body composition values.

(0.66 - 0.98), increased in four patients (1.05 - 1.52), and was normal in one. ICW/TBW ratio was reduced in ten of the patients. This, associated with the high ECW ratios, suggested that reduction in ICW maintained a relatively lower TBW than would have been anticipated from the increased ECW.

Body Cell Mass (BCM)

The mean derived BCM was 46.5% of body weight (range, 36.6 - 58.1%). BCM ratio was reduced in eight patients (0.67 - 0.98), increased in four patients (1.05 - 1.5), and unchanged in one. The changes in BCM were closely related to changes in ICW.

Total Body Solids (TBS)

Body solids were reduced in nine patients. This was due to reductions in body fat or fat-free solids, or both. In four patients (3, 6, 11, 13) TBS were not reduced although FFS ratios and BCM ratios were low, but body fat ratios were increased.

Fat-Free Solids (FFS)

Reduced FFS ratios were found in eleven patients ranging from 0.71 - 0.97. The remaining two patients had increased FFS ratios (1.04 and 1.22) (Table 4).

Total Body Fat (TBF)

Total body fat ranged from 4.7 - 27.7% of body weight (average 17.8%). Only two patients had body fat percentage below the third

percentile for age according to Cheek's normal values for children (Cheek, 1968). TBF ratios were reduced in seven patients (53.9%) and increased in six patients (46.1%). The changes in TBF ratios did not parallel FFS ratio deviations; six patients with low FFS ratios had increased TBF ratios. Body fat, in absolute values and as percentages of body weight, correlated significantly with both triceps and subscapular skinfold thickness (Table 5 and Fig.3).

TABLE 5

Skinfold	TBF (kg)		TBF (%BW)	
	r	p	r	p
Triceps	0.851	< 0.001	0.680	< 0.01
Subscapular	0.788	< 0.005	0.514	< 0.05

Table 5: Correlation between total body fat (TBF), in absolute amount (kg) and as percentage of body weight (BW), and triceps and subscapular skinfold thickness.

Fig. 4 shows the triceps skinfold thickness of the patients plotted against their height age on standard centile charts for normal British children (Tanner and Whitehouse, 1975). Nine of the thirteen children (5 boys and 4 girls) had values above the 50th centile and

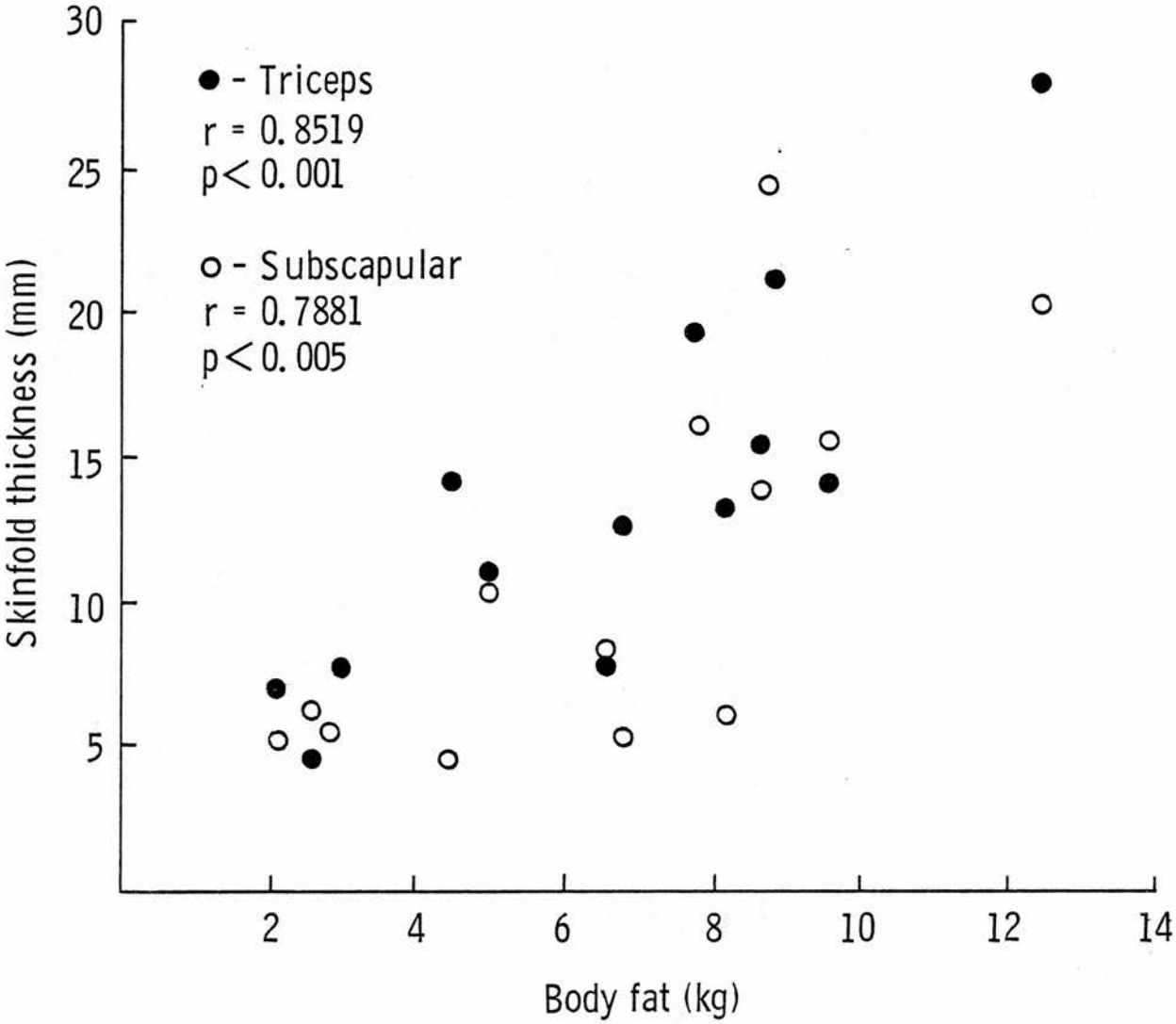

FIG. 3 Relationship between total body fat and triceps and subscapular skinfold thickness.

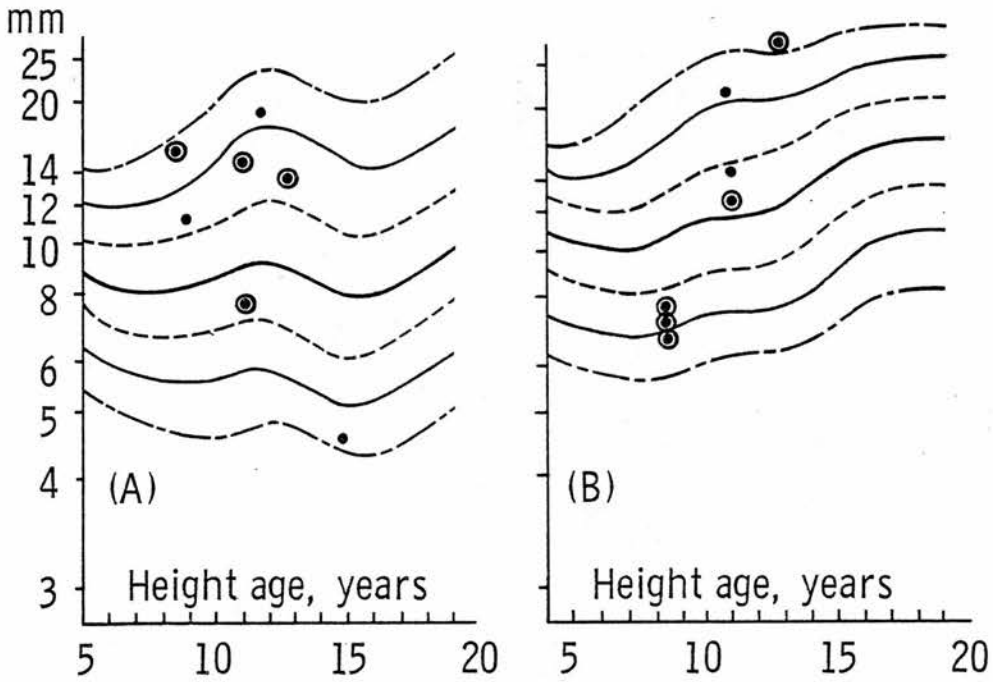

FIG. 4 Triceps skinfold thickness of boys (A) and girls (B) plotted against their height age on standard Centile charts (Tanner and Whitehouse, 1975). Circled values represent children with reduced body cell mass.

none was below the 3rd centile for height age. There was a tendency, in the boys, for prepubertal children to have the greater skinfold thickness. Values circled are from patients with reduced body cell mass. A similar pattern (not shown) was found with subscapular skinfold thickness.

Effect of Puberty on Body Composition

Comparisons of body composition changes in pubertal and prepubertal children are shown in Table 6. ICW, BCM and FFS ratios were significantly higher in pubertal than in prepubertal children. The deviations of these variables from the predicted normal values were significant in the prepubertal but not in the pubertal children indicating improvement in lean body mass with the onset of puberty.

ECW ratios were significantly higher in prepubertal compared with pubertal children but no significant difference was found in TBW ratios. TBF ratios though greater in prepubertal children, the difference did not reach significance ($P < 0.1$).

(B) OXYGEN CONSUMPTION

Basal oxygen consumption (VO_2) in litres per hour is recorded for each patient and control subject in Table 7. Brasel (1968), using the same method of measurement, found no sex difference in basal oxygen consumption for all indices used in the present study. Thus, the data for both boys and girls were combined for analysis.

TABLE 6

	$\frac{TBW_O}{TBW_P}$	$\frac{ECW_O}{ECW_P}$	$\frac{ICW_O}{ICW_P}$	$\frac{FFS_O}{FFS_P}$	$\frac{TBF_O}{TBF_P}$
Pubertal (8)	$1.07^{\pm}$ 0.06	$1.13^{\pm *}$ 0.13	$1.04^{\pm}$ 0.20	$0.94^{\pm}$ 0.14	$0.88^{\pm}$ 0.28
Pre-Pubertal (5)	$1.03^{\pm}$ 0.09	$1.46^{\pm **}$ 0.23	$0.78^{\pm **}$ 0.10	$0.78^{\pm **}$ 0.06	$1.25^{\pm}$ 0.42
Significance of Difference (P)	NS	<0.01	<0.05	<0.05	<0.1

Table 6: Body composition ratios in pubertal and prepubertal patients (mean $\pm$ 1 S.D. Abbreviations as in Tables 3 and 4.

* Observed values significantly different from predicted normal:

* $P < 0.05$ ** $P < 0.01$

TABLE 7

Patients		Controls	
No.	VO_2 (L/hr)	No.	VO_2 (L/hr)
1	10.83	1	7.90
2	11.06	2	6.76
3	10.38	3	6.32
4	12.41	4	8.78
5	7.75	5	8.36
6	10.25	6	9.86
7	9.80	7	8.15
8	7.84	8	10.34
9	7.07	9	8.35
10	9.01	10	14.95
11	10.17		
12	8.33		
13	10.39		
14	11.36		
16	8.70		

Table 7: Basal oxygen consumption (VO_2), in litres per hour, in patients and controls.

Basal oxygen consumption values in patients and normal subjects, expressed in relation to body surface area, height and body cell mass, are shown in Table 8 and Fig. 5. In the control group the average oxygen uptake per 100 cm height was similar to that reported by Brasel (1968) in a large group of normal children using the same method of

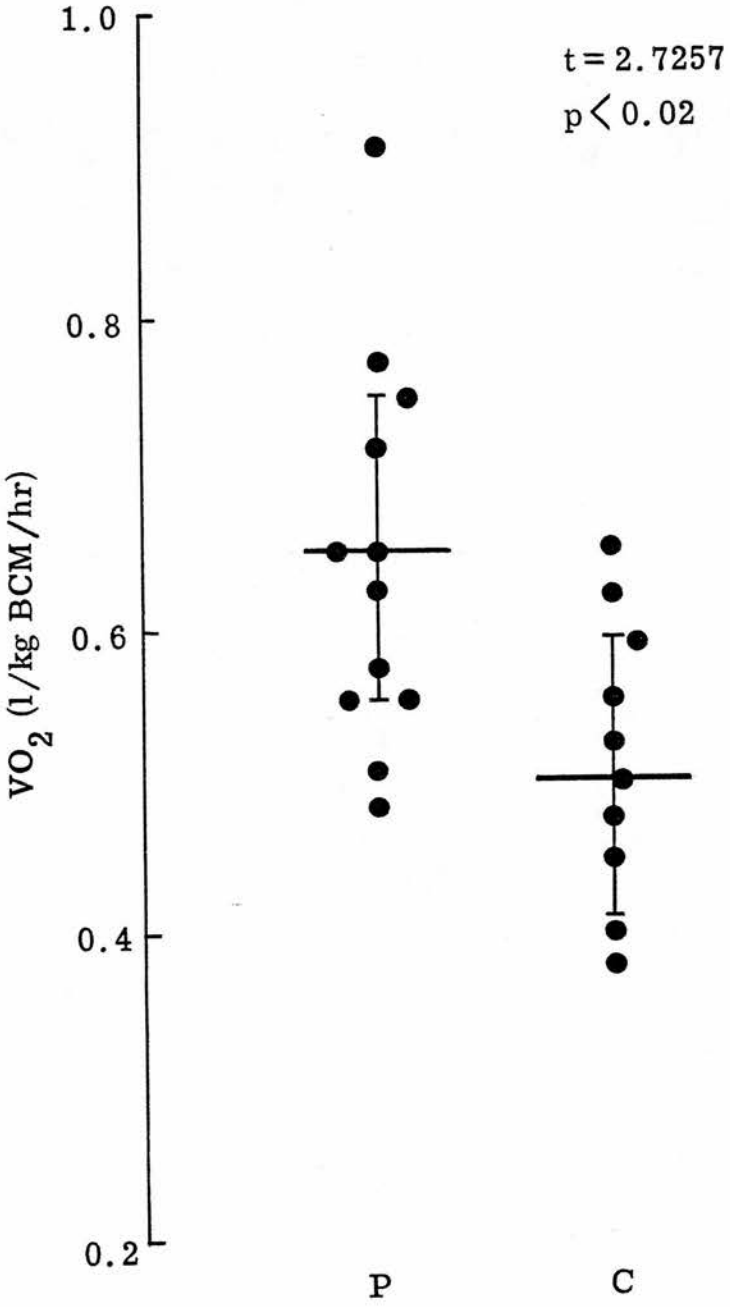

Fig. 5. Basal oxygen consumption (VO_2) in litres per hour per kg. body cell mass in patients (P) and controls (C).
Horizontal lines represent the mean ± 1 SD.

TABLE 8

	$\frac{VO_2(L/hr)}{Ht(m)}$	$\frac{VO_2(L/hr)}{SA\ (m^2)}$	$\frac{VO_2(L/hr)}{BCM(kg)}$
Patients (15)	6.93 ± 0.95	8.51 ± 1.11	0.66 ± 0.13
Controls	6.32 ± 1.2	7.37 ± 1.14	0.52 ± 0.09
t	1.371	2.431	2.725
p	<0.2	<0.05	<0.02

Table 8: Oxygen consumption (VO_2) in litres per hour in patients and controls expressed in terms of height (ht), surface area (SA) and body cell mass (BCM). Values are mean $\pm$ 1 S.D. Significance of difference was analysed by student 't'-test for unpaired data.

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measurement. Standard oxygen consumption, in terms of both body surface area and body cell mass, was significantly greater in patients compared to controls and although, in terms of height, it was higher in the patients than controls, the difference was not statistically significant.

In Table 9 the correlation coefficients between oxygen consumption and four reference standards in patients and controls are shown. As seen in the table weight was the best single variant for prediction of

TABLE 9

	Patients		Controls	
	r	p	r	p
VO_2 vs Wt.	0.697	< 0.01	0.809	< 0.005
VO_2 vs Ht.	0.546	< 0.05	0.720	< 0.02
VO_2 vs SA	0.659	< 0.02	0.799	< 0.01
VO_2 vs BCM	0.746	< 0.01	0.743	< 0.02

Table 9: Correlations between oxygen consumption in litres per hour (VO_2) and body weight (Wt.), height (Ht.), surface area (SA), and body cell mass (BCM) in patients and controls.

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oxygen uptake in the normal children, whereas body cell mass provided the highest correlation coefficient in the patients.

Relation of Oxygen Uptake to Body Cell Mass

Im Fig. 6 the oxygen consumption in litres per hour per kg body cell mass was plotted against body cell mass as a percentage of predicted normal, and it can be seen from the figure that the more wasted the child, the higher the oxygen uptake. Excluding the two patients with cystinosis (Nos. 13, 14), this inverse relationship becomes highly significant.

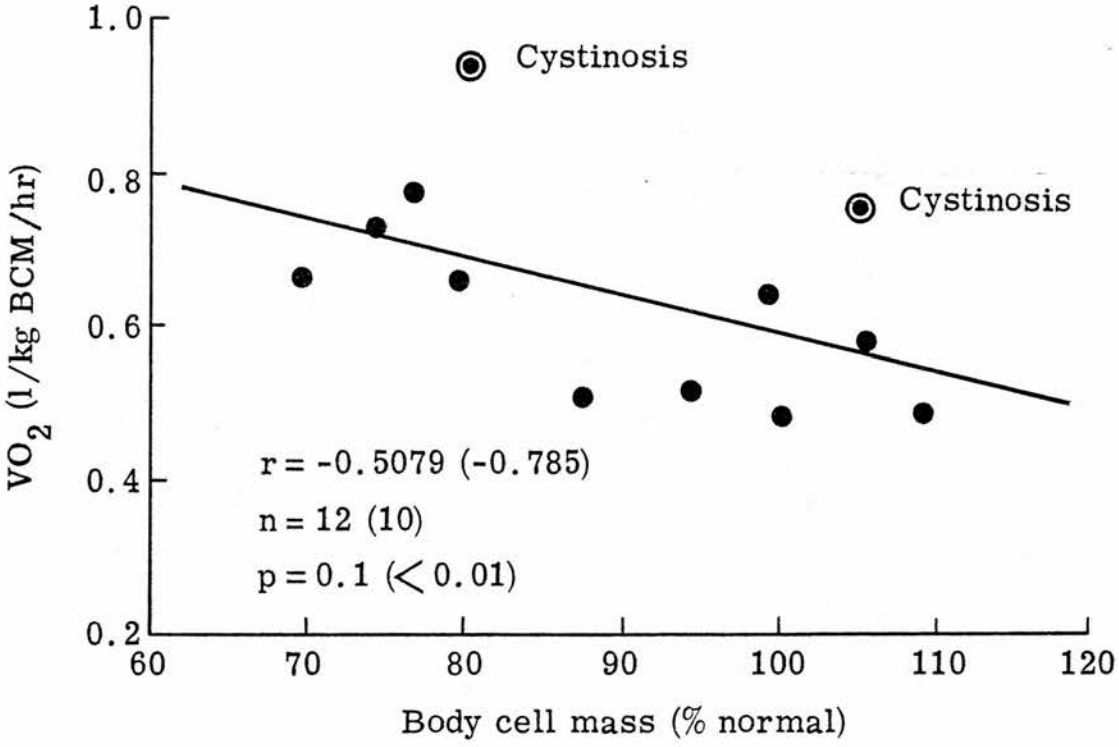

Fig. 6. Relationship between oxygen consumption in litres per hour per kg. body cell mass and body cell mass as a percentage of predicted normal.
Circled values represent the 2 cystinotic patients.

DISCUSSION

The impact of chronic renal failure on whole body composition in adult patients and the changes that occur on the institution of regular haemodialysis have been reported (Comty, 1968; Coles, 1972). Basically these reports have shown a relative increase in total body water largely due to an excess of extracellular fluid, a decrease in intracellular water and a variable reduction in fat-free solids and body fat. With the exception of one report on a 16 year old boy on haemodialysis (Wilson et al., 1973), there have been no studies on body composition in children with chronic renal failure. The results of the present study largely confirm the results obtained for adult patients and extend them to children.

The most marked and constant abnormality was ECV expansion. Although ECV expansion may indicate true overhydration, it is also a common compensatory response to a reduction in body cell mass as in children suffering from protein-energy malnutrition (McCance & Widdowson,1951).

Decreased ICW and the low ICW/TBW ratios indicated a loss of cell mass. Total body water (TBW) was only slightly increased despite marked increases in ECW. This suggested that extracellular overhydration and expansion due to loss of cell mass, on one hand, were offset by loss of cell mass and intracellular water on the other.

Eleven of the patients (84.6%) showed a decreased proportion of fat-free solids (FFS) when observed values were compared with predicted values (Table 4). FFS reflect body cell mass (Moore et al., 1963) and in the present study FFS ratios were in general closely related to

BCM ratios (Table 4) and there was a strong linear correlation between FFS and BCM ($r = 0.95$). The equations given by Moore et al. (1963) for the estimation of BCM and used in the present study is valid if the intracellular potassium concentration is normal, which is said to be the case even in extreme pathological disturbances (Moore et al., 1963). The high correlation coefficient (0.95) obtained from the regression on FFS, derived from the variable hydration of fat-free body, gives credibility to this assumption.

An absolute increase in body fat occurred in six patients (46.2%) despite reduction in BCM and 69% of the patients had skinfold thickness above 50th centile for height age. This is contrary to the previously held view that chronic renal failure is associated with reduction in total body fat (Comty, 1968; Coles, 1972). Such observations were based on studies of patients who were undoubtedly malnourished. However, Comty (1968) has noted an absolute and relative increase in body fat with accompanying loss of fat-free solids in 36% of her non-dialysed patients and Coles (1972) reported an increase in body fat following regular haemodialysis treatment. Although none of the available methods for estimation of body fat are entirely satisfactory (Forbes, 1962), the body water method using Moore's nomogram of variable hydration of fat-free body (Moore et al., 1963) correcting for the possible deviation from normal of the water content of fat-free body in these patients seemed justified. Body fat estimated by skinfold thickness agreed well with simultaneous estimation by body water method in children (Brook, 1971) and the strong linear correlation between body fat and skinfold thickness found in the present study supports the validity of the method.

The gain in body fat and the loss of FFS observed in some of these children whose dietary intake of energy and protein were adequate suggest a defect in intermediary metabolism with a decrease in the utilisation of endogenous fat for energy production and a decrease in net protein anabolism. This is in keeping with the observations made in the preceding study (Study 2) and gives support to the hypothesis made that the prime defect seems to be a failure of utilization of conventional sources of energy substrates consequent upon tissue insulin resistance, among other things, leading to increased body protein depletion.

The finding of significantly greater deficit of body cell mass in the prepubertal compared with pubertal children is interesting and may help to explain the better growth performance of the pubertal children observed in Study I. Analysis of growth in a large number of children on haemodialysis in Europe showed similar results (Chantler et al., 1977). Therefore, the decreased growth velocity of children in renal failure may be associated with decreased cell mass and it is possible that the increased androgen production of pubertal children is associated with an increased growth velocity and body cell mass. With this in mind, we have treated a group of prepubertal boys on haemodialysis for chronic renal failure with an anabolic steroid (oxandrolone) with improvement in body composition and growth velocity (Carter et al., 1978).

Oxygen consumption

Standard oxygen consumption, which reflects basal energy expenditure,was found to be higher in these patients than in controls

matched for height and studied under the same conditions, both in terms of body surface area and body cell mass. Whether a true hypermetabolism existed in these children was difficult to ascertain. An increase in oxygen consumption per unit body cell mass might have been merely a reflection of a relative increase of the metabolically active tissues (internal organs) and a reduction in the proportion of tissues of low metabolic activity (fat and muscle). However, this seemed unlikely as body fat was found to be normal or even increased in most of the children and when height, which reflects organ size (Holliday, 1971), was used as reference standard, the difference in oxygen uptake persisted although it did not reach the conventional level of significance.

Basal energy expenditure in children recovering from protein energy malnutrition is increased and is directly proportional to the degree of protein depletion (Montgomery, 1962). Brook and Ashworth (1972) have suggested that the marked increase in oxygen consumption during recovery from malnutrition is related to the high energy requirements for protein synthesis. This may account for the high energy costs of growth in previously malnourished children (Ashworth et al., 1968). Similarly Kein et al. (1978) have recently demonstrated an increase in BMR in children recovering from burn injury and suggested that the basal energy expenditure is directly associated with energy needs for whole protein synthesis and breakdown. It is conceivable that the increased oxygen consumption of the children in the present study was due to increased protein turnover and were, in this respect,

not unlike those children recovering from malnutrition or catabolic injury. The inverse relationship between oxygen uptake and the per cent deficit in body cell mass suggests that this may indeed be the case. Energy and protein requirements must therefore be increased to support the rate of protein synthesis that would equal or exceed protein degradation for net protein gain. However, it must be recognised that the present data is too limited to permit exploration of the relationship between basal oxygen consumption and protein turnover status in these children and, therefore, the foregoing conclusion must be regarded as tentative. Obviously, further study will be needed to, (a) determine if the increase in basal metabolism is due to a greater amount of metabolically active tissue or to a change in the metabolic activity of the muscles and organs and (b) to assess the relationship between basal energy expenditure and whole body protein synthesis. Such studies will help to determine maintenance energy requirements for net protein anabolism in these patients.

CHAPTER 10

STUDY IV

EFFECT OF DIET ON PLASMA LIPID LEVELS

Concern is developing that an accelerated rate of atherosclerosis is occurring in adult patients on regular haemodialysis and is resulting in premature deaths from cardiovascular disease (Lindner et al., 1974). In view of this and the relationship between hyperlipidaemia and cardiovascular disease shown in normal population (Kannel et al., 1971; Carlson and Böttinger, 1972), the findings of increased plasma triglycerides and cholesterol levels in the group of children under study (see Study I) were disturbing. Uraemic patients are at risk of for several other reasons including hypertension, hyperuricaemia and carbohydrate intolerance.

Although the exact mechanism of the hyperlipidaemia is not well understood, it is obviously of practical importance to attempt to reduce the lipid levels in these patients, particularly the cholesterol levels, as their reduction is known to improve mortality from cardiovascular disease (Miettien, 1972). The use of clofibrate is contraindicated in end stage renal failure as it is excreted by the kidney. Dietary treatment is, therefore, of prime importance. In this group of patients the principle therapeutic step would be to reduce carbohydrate and to increase the polyunsaturated/saturated fatty acid ratio (P/S ratio) in the diet. This in normal subjects lowers the plasma cholesterol and plasma triglycerides.

The purpose of this study was (1) to further evaluate the relationship between dietary intakes of carbohydrate, fat and protein

and plasma lipid levels and (2) to determine whether a diet low in saturated fats and high in polyunsaturated fats results in lower levels of plasma lipids or not. If it does, then long term treatment on such a diet would be a reasonable measure to reduce at least one of the risk factors in these young patients.

METHODS

Patients and Procedure

Clinical data and management of the patients were as described in Study I.

Two periods of observation were allocated. During the first period of one year, all the patients (16) were allowed free diet and an energy supplement based on a glucose polymer (caloreen) containing 17 Kj/g and and saturated fat in the form of double cream (4 Kcal/ml).

During a second period of one year the energy supplement was altered in that the saturated fat was replaced by a recipe high in polyunsaturated fats based on sunflower seed oil which has a high energy density (37 Kj /g). The supplement was designed to increase the polyunsaturated fats/saturated fats ratio (p/s) in the diet. Detailed description of the recipe is given in Table I, Appendix (C).

Although the children were not obliged to follow a rigid dietary regime and took the energy supplements in varied amounts in both periods, adherence to the prescribed supplement was considered essential and they were strictly supervised by a trained dietician. The dietary intakes

were monitored by means of a weighed dietary intake as described in Chapter 5. At the end of each period, plasma triglycerides (TG) and cholesterol (CHOL) concentrations were determined by the methods outlined in Chapter 6.

For the purpose of this study, the intakes recorded closest to blood sampling were used for analysis. During the second period four patients did not tolerate the oil supplement and had nausea and vomiting and three patients did not adhere to it and were excluded from the study. Only nine patients completed the study satisfactorily and analysis, therefore, was restricted to these nine patients.

The patients acted as their own controls and plasma lipid levels were also compared to normal control values shown in Study I.

RESULTS

Clinical data of the patients are shown in Table I of Study I.

Nutrient Intakes

The mean intakes of total energy, carbohydrates, fats and protein in absolute amounts and as a percentage of recommended daily allowance (% RDA) for height age in the first period (p_1) and second period (p_2) are shown in Table I. The proportions of energy derived from carbohydrates, fats and protein are also shown in the table. Data for individual patients is presented in Table 2 of Appendix (C). Total energy, carbohydrate, total fat and protein intakes were similar in both periods. In the second period, the percentage of energy derived from carbohydrate was significantly higher ($p < 0.05$) (Fig. 1). The percentage from fat was higher but not significantly ($p < 0.1$), and the percentage from protein was significantly lower ($p < 0.02$). The p/s ratio in the diet was significantly higher in the second period compared to the first period ($p < 0.05$) (Fig. 1).

Plasma Lipid Levels

Figure 2 shows the plasma triglyceride and cholesterol levels in the first and second period and in relation to control values. Plasma triglyceride and cholesterol concentrations were significantly elevated in both periods compared to controls. In the second period mean plasma TG was higher but not significantly ($p < 0.1$), whereas mean plasma cholesterol was significantly lower ($p < 0.05$).

TABLE I

	Total energy		Carbohydrate		Fat		Protein		Carbohydrate (% total energy)	Fat (% total energy)	Protein (% total energy)
	Kj/d	%RDA	gm/d	%RDA	gm/d	%RDA	gm/d	%RDA			
P_1	$9577^{\pm}$ 2448	$100.4^{\pm}$ 29.7	$244^{\pm}$ 78	$92,7^{\pm}$ 35.8	$124^{\pm}$ 41	$116.6^{\pm}$ 39.5	$59.8^{\pm}$ 24	$104.4^{\pm}$ 46.7	$40.6^{\pm}$ 10	$48.6^{\pm}$ 9.2	$10.5^{\pm}$ 2.7
P_2	$9956^{\pm}$ 4078	$96.8^{\pm}$ 34.6	$263^{\pm}$ 62	$93.9^{\pm}$ 19.8	$120^{\pm}$ 74	$107.2^{\pm}$ 63.1	$56.1^{\pm}$ 28	$93.8^{\pm}$ 50.5	$48.6^{\pm}$ 12.6	$42.9^{\pm}$ 10.6	$8.2^{\pm}$ 2.3
t^*	-0.354	+0.317	-0.726	-0.103	+0.211	+0.579	+0.574	+0.891	-2.478	+1.906	+3.336
p	NS	NS	NS	NS	NS	NS	NS	NS	< 0.05	< 0.01	< 0.02

Table 1: Dietary intakes, expressed in absolute amounts per day and as percentage of recommended daily allowance (RDA) for height age, in the first period (p_1) and the second period (p_2). Percentages of total energy derived from carbohydrates, fats and protein are also shown. Values are mean $\pm$ 1 S.D.

* significance of difference was analysed by paired t-test.

NS = not significant.

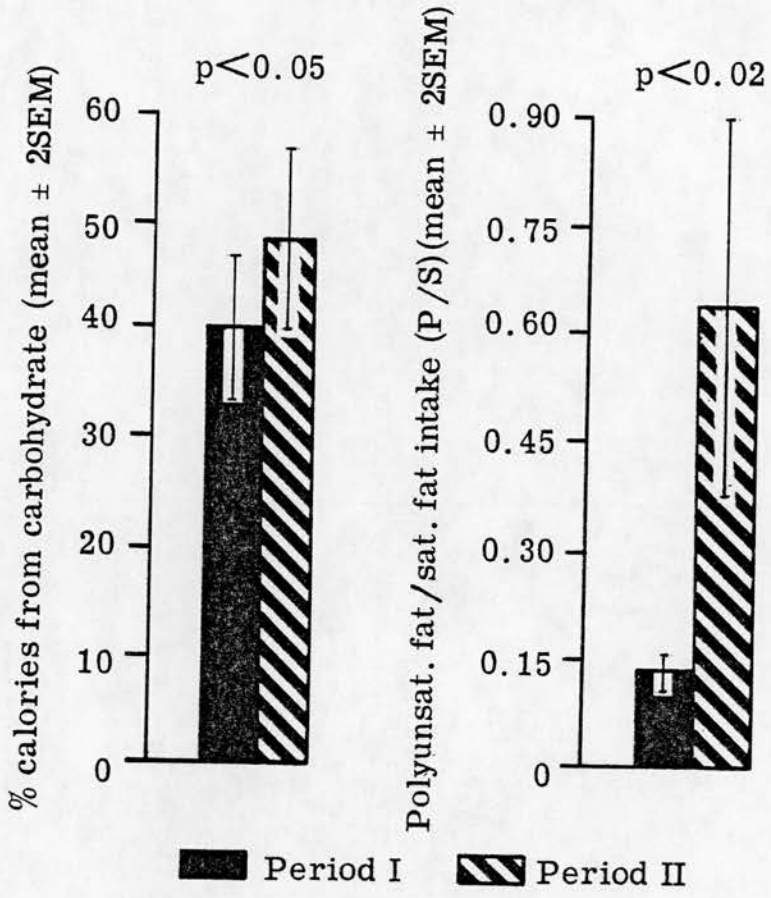

Fig. 1. Carbohydrate intake (% of total energy) and polyunsat. to sat. fat intake ratio in first and second period. Mean ± 2 SEM

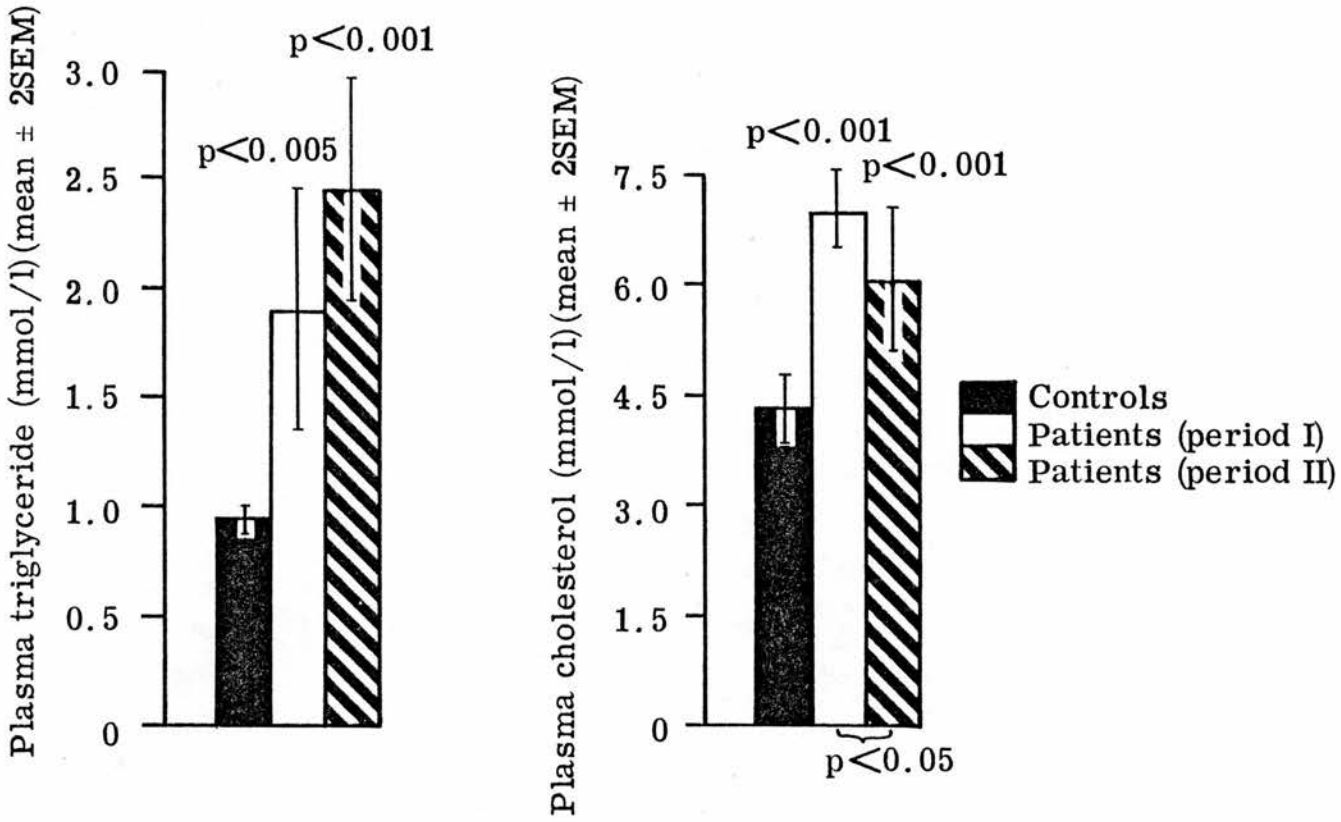

Fig. 2. Mean ± 2SEM of plasma triglyceride and cholesterol concentrations in the first and second period and in control.

Relationship between diet and Plasma Lipids

In Table 2, the correlation coefficients between dietary intakes and plasma TG and CHOL levels in the first period (p_1) and the second period (p_2) are shown. In both periods there was an inverse relationship between fat intake and TG levels ($p < 0.01$ and $p < 0.02$, respectively) and in the second period an inverse relationship between total energy intake and TG levels was present ($p < 0.05$). In both periods the percentage of energy from carbohydrate correlated directly with plasma TG levels ($p < 0.02$ and $p < 0.05$ respectively). In the second period plasma TG correlated inversely with protein intake ($p < 0.02$). Plasma CHOL did not correlate with diet in the first period but in the second period an unexpected inverse relationship with carbohydrate intake was noted.

TABLE 2

	Triglycerides	Cholesterol
Energy % RDA		
P_1	-0.526	-0.3911
P_2	-0.7412*	-0.17
Fat % RDA		
P_1	-0.818***	-0.3367
P_2	-0.7801**	-0.0317
Carbohydrate % RDA		
P_1	0.0059	-0.4004
P_2	-0.3490	-0.6808 *
Protein % RDA		
P_1	-0.637	-0.2849
P_2	-0.7973**	0.0022
Fat % energy		
P_1	-0.7209*	-0.0349
P_2	-0.6947*	0.0323
CHO % energy		
P_1	+0.7331**	0.0119
P_2	+0.706*	-0.0476
Protein % energy		
P_1	-0.3251	0.0305
P_2	-0.6859*	0.0678

Table 2: Correlation coefficients (r) between plasma lipids and dietary intake, expressed as % recommended for height age (RDA), in first period (p_1) and second period (p_2).

* $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$

DISCUSSION

As pointed out in Chapter 3, patients with chronic renal failure commonly develop type IV hyperlipidaemia which does not improve but is, if anything, accentuated on haemodialysis. It has already been noted (Study I) that the hyperlipidaemia of uraemia is as much a problem for children as it is for adults and the results of the present study indicated that it is a persistent abnormality.

Although recent literature (Bagdade et al., 1978) suggests that defective clearance of the triglyceride-rich very low density lipoprotein (VLDLP) from plasma is the major cause of the hypertriglyceridaemia in uraemic patients, increased hepatic production of triglycerides, as a result of hyperinsulinism and carbohydrate intolerance, plays a contributory role in its pathogenesis (Bagdade, 1970; Sanfellippo et al., 1977). When the proportion of energy from carbohydrate increased in the second period, compared to the first period, plasma TG levels also increased. This and the persistently significant correlation found between plasma TG and energy derived from carbohydrate suggest that dietary carbohydrate is a factor in the pathogenesis of the hypertriglyceridaemia. Others (Pennesi et al., 1976; Broyer et al., 1976) have reported similar results in children on regular haemodialysis. Although it will not correct the abnormality, reduction of precursors of triglycerides such as dietary carbohydrates may therefore effect a decrease in plasma TG levels in this respect. Sanfellippo et al. (1977) have reported a prompt reduction in plasma TG levels in their uraemic patients following a relatively short period of time (eleven days) on a diet low in

carbohydrate, possibly secondary to a decrease in insulin concentrations elicited by that diet.

Prospective epidemiological and other studies (Kannel et al., 1971; Dayton et al., 1970) have shown that hypercholesterolaemia is an independent major risk factor in cardiovascular disease. Therefore, the finding of a high plasma cholesterol level in these children awaiting renal transplantation is disturbing and therapeutic approaches for amelioration of this abnormality are needed. Although no direct correlation between fat intake and plasma cholesterol could be demonstrated in this study, the significant increase in the polyunsaturated/saturated fats ratio (p/s) in the diet in the second period was associated with a significant decrease in plasma cholesterol levels. In normal individuals an increase in p/s ratio lowers both plasma cholesterol and plasma triglycerides. The absence of a lowering effect on plasma TG levels in the patients suggests different mechanisms for the two abnormalities. However, the purpose of this study was not to define the discrete dietary variable responsible for hypercholesterolaemia in these patients. Rather an attempt was made to determine whether plasma cholesterol levels in these patients would respond to relatively simple dietary modification.

The disparity between dietary intake and requirements is often greater in uraemic children than in adults, because of their increased energy needs in relation to body size and the need to ensure adequate nutrition in children with renal failure has already been emphasized. The results of this study suggest that restriction of dietary energy,

protein and fat may exacerbate rather than ameliorate hypertriglyceridaemia in uraemic children but dietary carbohydrate is associated with elevation in plasma TG concentration, and that an increase in p/s ratio in the diet reduces plasma cholesterol level. However, further studies of the effect of various dietary constituents on plasma lipids are required, but in the meantime it seems reasonable to try to ensure an adequate intake of total energy and protein. Regular diet surveillance is important and for those children with raised lipid levels, the proportions of carbohydrate and saturated fat in the diet need to be controlled.

In conclusion, therefore, dietary management of hyperlipidaemia in uraemic children ensuring adequate energy intake, primarily from polyunsaturated fat, and reducing carbohydrate intake, may be important as a primary longitudinal approach to reduction of the increased atherosclerotic risk in these patients.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUSIONS

This thesis did not aim to identify a single cause for the growth retardation of children with chronic renal failure but rather to obtain a general view of nutritional, hormonal and metabolic disturbances, their interrelationships and relevance to growth. The observations made appear helpful in further understanding the pathogenesis of this problem, and provide information which may be important in the strategy for nutritional therapy in these children.

Linear growth rate of most patients was subnormal with variable delay in skeletal maturation and no catch-up growth was observed. In general pubertal children grew better than prepubertal children and continued to grow at an age when growth would be expected to cease in normal children. However the data indicated that skeletal maturation (bone age) advanced more rapidly than did linear growth (height age); consequently growth potential was progressively lost. Thus, attempts to maximise growth in early childhood before the institution of dialysis by adequate nutritional therapy, careful control of renal osteodystrophy, acidosis, hypertension and anaemia is very important. Regular assessment of skeletal maturation and height age is important, and when growth potential is clearly reduced, early renal transplantation is indicated to improve ultimate height.

Puberty, particularly in boys, was delayed and the results indicated that this might be related to gonadal dysfunction at least in relation to chronological age. However the delay in the initiation of sexual

development might also be the result of a hypothalmic-pituitary defect secondary to uraemia. Hyperprolactinaemia might also be implicated in the pathogenesis of the delayed puberty but further studies are required.

Energy and protein intake of most patients appeared adequate in relation to normal children of the same height age, but data obtained from basal energy expenditure assessment and the relationships between metabolic hormones and energy substrates would suggest that there was an additional demand for energy over and above that required in normal children. Studies in acutely uraemic children (Abitbol and Holliday, 1976) and in chronically uraemic rats (Mehls et al., 1978) support the concept of increased energy requirements in uraemic children. The reason for the increase in energy requirements is obscure and further studies are required to examine the efficiency of energy utilization for protein synthesis in these children.

It was speculated that the prime cause of energy deficit was failure of utilization of glucose by cells and decreased fat availability for energy production as a result of peripheral insulin resistance and hyperinsulinaemia. It was further speculated that the requirement of energy from protein was increased leading to an increase in essential amino acid catabolism, an increase in alanine release from muscle, enhanced gluconeogenesis and body protein depletion. An increase in protein catabolism and/or a decrease in protein anabolism might therefore be secondary to the need

of energy production from protein. Thus a failure of normal energy metabolism or an increase in the energy cost of metabolism will reduce protein anabolism and presumably growth. Clearly the need for adequate energy and protein intake to fulfil the requirements is very important.

The metabolic and hormonal changes described in this thesis were in many respects similar to the changes seen in other catabolic states such as sepsis, burns or major trauma. The metabolic effects of uraemia tend to alter the balance between protein anabolism and catabolism in favour of catabolism and produce hormonal changes which reflect and aid this alteration.

The studies on body composition demonstrated that intracellular water or cell mass was reduced especially in the prepubertal children. In spite of the reduction in cell mass some children were obese with an increase in body fat derived from body water measurements or assessed from skinfold thickness. Energy supplements, as carbohydrate, may therefore increase body fat without increasing muscle mass. The pubertal children grew better and had more normal body composition than prepubertal children, and a positive correlation existed between growth velocity and plasma sex hormone levels in the pubertal but not in the prepubertal children. It was speculated that the difference in body composition was determined by a lower anabolic drive before puberty and it was suggested that anabolic steroids might have a place in the treatment of prepubertal children with

chronic renal failure. This awaits further studies.

Hyperlipidaemia was common in these children and the results indicated that the hypertriglyceridaemia was associated with the proportion of carbohydrates in the diet and increasing the poly-unsaturated/saturated fat ratio in the diet had a lowering effect on the elevated plasma cholesterol levels. In view of the high incidence of atherosclerosis and premature deaths reported in adults on maintenance haemodialysis; it would seem desirable to reduce dietary carbohydrates and to supply significant proportion of dietary energy as polyunsaturated fats.

Many biochemical, metabolic and hormonal changes were demonstrated in the present study, but it proved difficult to relate the abnormalities found to growth, perhaps because growth is influenced by multiple factors. It was unlikely that a single factor was responsible for the growth failure in these children. A positive relationship was found between growth velocity and the plasma levels of the branched chain amino acids, valine and leucine. Growth may have been adversely affected by the reduction in the availability of the branched-chain amino acids for protein synthesis, or the low levels may reflect energy deficit, secondary to uraemia, which in turn affected growth. It was suggested that the provision of extra essential amino acids or their ketoanalogues in the diet might improve protein synthesis and growth if nitrogen toxicity is reduced, by lowering protein intake, and adequate energy intake ensured. In this respect,

short-term results reported recently by Giordano et al. (1978) were encouraging but long-term studies are awaited.

Many statistically significant correlations were found between the large numbers of variables studied. It is stressed, however, that such correlations do not prove a cause and effect relationship, particularly in a disease like uraemia which affects a wide spectrum of interdependent and independent metabolic activities. They are only a guide to the direction of future research.

The results suggested that the cause of growth retardation of children with chronic renal failure is multifactorial and is a cumulative effect of complex and interrelated metabolic and hormonal derangements. A solution to this problem will most likely be forthcoming only after the basic cellular defects in energy and protein metabolism are elucidated. Means of improving the insulin resistance and altering the balance between anabolism and catabolism should also be studied.

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A P P E N D I C E S

APPENDIX (A)

TABLE I

Case No.	Plasma Transferrin (mg/dl)			Plasma Albumin (g/dl)			Plasma C_3 (% RNS)		
	S	M	E	S	M	E	S	M	E
1	152	145	160	4.2	3.6	4.6	-	106	-
2	230	240	220	4.1	4.5	4.0	-	94	-
3	280	170	300	4.4	4.5	4.2	-	132	-
4	340	155	250	4.4	4.5	4.3	-	100	-
5	390	150	270	3.8	4.1	4.2	-	106	-
6	160	170	240	4.2	3.8	4.4	-	75	-
7	200	180	190	4.4	3.9	4.0	-	78	-
8	120	180	145	3.5	3.6	3.4	-	87	-
9	160	110	240	3.7	4.0	5	-	132	-
10	210	170	130	4.3	4.1	4.1	-	94	-
11	170	320	270	3.8	4.5	4.3	-	86	-
12	145	280	195	4	3.8	4	-	115	-
13	120	350	190	4.2	4.0	3.5	-	116	-
14	310	320	210	4.9	5.3	4.5	-	116	-
15	195	320	250	4.0	4.4	4.3	-	92	-
16	160	360	260	3.9	4.0	3.9	-	110	-

TABLE 2

Case No.	Plasma Insulin (mU/l)			Plasma growth hormone (mU/l)			Plasma Cortisol (nmol/l)		
	S	M	E	S	M	E	S	M	E
1	-	14	16	12	37	22	-	309	234.6
2	14	26	18	10	12	7	496.8	430.6	607.2
3	-	18	12	-	11	11	-	270.5	358.8
4	15	12	17	13	15	17	648.6	449.9	334
5	16	22	18	4	5	9	552	665.2	386.4
6	21	18	11	14	7	15	229	276	262
7	-	15	16	-	9	7	-	507.8	394.7
8	20	18	8	11	10	18	717.6	543.7	240
9	17	14	16	23	26	25	389.2	505	634.8
10	21	16	18	7	2	7	626.8	364.3	524.4
11	37	24	20	19	21	14	662.4	623.7	593.4
12	19	12	12	27	17	19	524.4	358.8	543.7
13	21	28	25	28	20	38	516	449.9	320.2
14	12	31	18	10	7	18	347.8	303.6	510.6
15	27	45	27	12	6	8	703.8	469.2	441.6
16	14	10	18	11	4	9	342.2	284.3	262.2

TABLE 3

Case No.	T_4 (nmol/l)			T_3 (nmol/l)			TSH (mu/l)			T_3RU (%)			FTI (%)		
	S	M	E	S	M	E	S	M	E	S	M	E	S	M	E
1	90.1	84.9	78.5	1.66	1.92	2.3	1.9	1.3	2.4	120.8	116.5	119.7	74.6	72.9	65.6
2	118.4	111.9	113.2	2.1	1.96	2.6	2.1	2.2	2.8	96.3	99.4	95	122.9	88.8	119.1
3	81.1	70.8	91.4	2.7	2.1	3.2	1.5	1.7	1.8	109.3	98.6	102.3	74.2	71.8	89.3
4	115.8	111.9	141.6	3.0	2.6	2.83	3.1	2.8	2.6	107.8	105.9	106	107.4	105.6	133.6
5	93.9	105.5	86	1.49	1.3	1.35	2.8	2.5	1.6	92.6	93	90.8	101.4	113.4	94.7
6	119.5	119.7	99,1	2.77	2.86	2.57	1.1	0.9	1.3	111.8	111.7	109.2	106.9	107.2	90.7
7	117.1	104.2	98	2.8	2.7	1.8	3.8	6.3	3.6	107.4	106.1	98.8	109	98.2	99.2
8	74	115.8	73.3	1.25	1.58	1.54	1.7	2.8	2.1	101.4	166.4	110.3	72.9	99.5	66.4
9	127.4	96.5	105.5	2.8	1.89	2.1	2.4	2.6	1.9	109.3	110	109.2	116.5	87.7	96.6
10	83.6	77.2	81.1	2.7	2.51	2.8	2.2	2.5	3.0	107.3	109.4	103.9	77.9	70.6	78.1
11	126.1	115.8	110	1.2	1.1	1.3	0.8	1.1	1.3	98.0	97.8	101.5	128.7	118.4	108.4
12	124.8	114.5	-	2.8	1.9	-	2.7	3.6	-	108	113.6	-	115.5	100.8	-
13	90.1	101.6	86	2.4	2.1	3.3	> 40	24.5	> 40	121.1	121.3	129	74.4	83.7	66.7
14	105.5	113.2	120.9	2.67	2.65	3.2	2.8	26.9	> 40	111.5	113	114.9	94.6	89.9	74.8
15	-	79.8	117.1	-	2.23	3.00	-	0.9	1.4	- .	102.9	72.8	-	77.6	150.9
16	104.2	77.2	109.4	2.29	2.51	2.18	2.1	2.4	2.6	109.1	107.9	116.0	95.5	71.5	94.3

TABLE 4

Case No.	Blood Glucose (mmol/l)		
	S	M	E
1	-	4.18	5.33
2	5.11	4.89	5.0
3	-	5.17	5.22
4	5.28	5.1	4.84
5	4.84	4.78	-
6	4.95	4.78	4.56
7	5.06	4.95	-
8	-	5.28	4.56
9	3.96	5.0	4.89
10	5.06	4.95	5.22
11	6.32	6.05	-
12	4.78	5.0	-
13	5.06	5.06	6.32
14	5.72	4.67	5.61
15	4.84	5.06	-
16	4.29	4.45	4.62

TABLE 5

Case No.	TG (mmol/l)			CHOL (mmol/l)			NEFA (μeq/l)			Serum Glycerol (mg/dl)			Electrophoretic Pattern
	S	M	E	S	M	E	S	M	E	S	M	E	
1	-	1.375	0.687	-	4.46	4.77	-	1327	562	-	0.76	0.90	Normal
2	2.775	3.54	2.53	6.45	7.14	5.16	549	1026	610	107	1.5	0.81	11b
3	-	0.725	1.125	-	7.6	6.42	-	606	622	-	1.1	0.95	Normal
4	1.475	1.675	1.75	6.70	6.8	5.93	556	928	672	1.6	1.2	0.78	IV
5	1.50	1.55	-	6.73	6.3	-	747	652	-	0.83	0.81	-	IV
6	2.40	1.525	1.325	6.65	7.1	7.19	513	831	653	0.85	1.6	1.10	IV
7	1.75	1.70	-	7.06	5.42	-	510	478	-	0.98	0.65	-	IV
8	-	1.75	1.125	-	4.56	4.54	-	597	710	-	0.84	0.86	IV
9	2.70	2.72	1.86	5.70	5.34	5.54	604	717	459	1.2	0.86	1.0	IV
10	2.886	3.20	2.737	6.01	7.22	6.19	510	577	632	0.78	0.9	0.95	IV
11	1.937	1.675	-	5.34	4.85	-	430	490	-	0.55	0.7	-	IV
12	1.187	1.25	-	5.26	6.10	-	661	696	-	1.02	0.7	-	Normal
13	2.162	2.237	2.325	7.68	8.85	7.79	473	724	420	0.72	0.7	0.9	11b
14	1.45	2.675	1.925	6.96	7.40	6.86	518	449	540	0.80	0.82	0.69	IV
15	1.375	3.55	2.53	5.34	4.80	5.42	570	620	640	1.7	1.2	1.5	IV
16	0.675	1.14	0.737	6.29	6.50	6.37	650	565	388	1.10	0.80	1.01	Normal

APPENDIX B

TABLE I

Hormonal and metabolic responses to iv glucose.

Blood Glucose (mmol/l)

Case No.	Time Mins 0	5	10	15	20	30	40	50	60	90	Kg
1	4.18	9.29	8.14	7.42	6.43	5.01	4.18	4.07	4.12	4.23	1.09
2	5.83	12.32	11.0	8.36	7.15	5.61	5.28	5.06	5.28	5.5	0.709
3	5.72	13.31	11.55	10.39	10.12	9.02	3.14	7.04	6.71	5.72	1.06
4	4.62	10.5	9.62	8.58	6.82	5.55	4.56	4.02	3.91	4.62	1.43
5	4.84	12.65	10.34	8.63	7.48	6.27	5.28	5.11	5.06	5.17	0.99
6	5.06	10.78	9.68	9.13	8.25	7.15	5.94	5.28	4.51	4.4	1.51
7	4.95	10.94	8.19	6.38	5.5	4.51	4.18	4.29	4.51	4.62	0.46
8	5.28	11.77	10.67	9.24	9.13	7.04	6.54	5.83	5.55	5.94	1.18
9	4.89	10.45	8.8	7.64	7.09	6.16	5.22	4.78	4.29	4.56	1.25
10	5.22	10.89	9.68	8.91	7.70	6.98	6.49	5.33	5.33	5.28	1.00
11	6.32	13.64	11.88	10.72	10.12	9.13	8.14	7.37	7.15	6.27	0.93
12	4.78	10.34	9.46	8.19	7.20	5.88	4.84	4.45	4.12	4.78	1.39
13	6.32	12.70	10.56	9.73	8.74	7.15	6.16	5.56	5.28	5.39	1.27
14	5.61	13.64	10.56	9.68	8.14	6.65	6.05	6.27	5.77	6.27	0.74
15	5.17	13.75	11.66	10.12	8.03	7.26	6.71	5.61	5.28	4.73	1.09
16	4.62	11.27	10.28	8.91	7.81	6.60	5.77	4.89	4.51	4.23	1.39

TABLE I (continued)

Plasma IRI

(mU/l)

Case No.	0'	5'	10'	15'	20'	30'	40'	50'	60'	90'
1	14	55	32	22	45	18	12	10	14	6
2	18	60	45	27	30	30	33	28	21	30
3	12	58	37	57	42	42	51	34	21	31
4	3	50	29	27	18	15	17	14	16	17
5	18	46	36	33	30	24	24	27	30	30
6	11	44	33	30	23	23	21	19	19	14
7	16	37	63	38	30	20	13	12	18	19
8	12	27	18	24	18	15	15	19	16	12
9	14	43	30	40	34	27	23	16	19	17
10	18	73	45	35	30	23	17	14	19	16
11	37	108	60	90	82	77	66	61	46	36
12	21	55	41	39	33	30	21	15	20	11
13	25	61	52	46	50	38	29	30	31	36
14	18	72	27	22	28	21	16	17	19	14
15	22	123	63	38	30	24	23	22	21	19
16	8	48	28	20	20	17	10	10	17	11

TABLE I (continued)

Plasma GH
(mU/l)

Case No.	0'	5'	10'	15'	20'	30'	40'	50'	60'	90'
1	37	40	46	49	46	32	14	14	-	8
2	7	8	6	6	6	7	13	14	14	13
3	24	17	14	13	14	14	9	10	10	29
4	9	11	15	17	32	-	41	24	16	14
5	9	11	9	10	9	10	10	8	10	-
6	15	25	22	27	62	94	72	72	30	15
7	7	7	8	8	7	6	7	6	6	7
8	10	10	11	10	12	10	9	8	6	9
9	35	78	96	98	98	90	90	92	80	27
10	7	8	9	7	9	12	13	11	12	7
11	21	32	25	30	30	60	61	32	24	17
12	27	57	60	64	60	64	64	62	60	50
13	38	55	62	62	60	43	18	12	8	6
14	32	11	10	11	10	10	8	8	18	18
15	5	9	7	8	5	8	19	34	18	20
16	9	11	10	10	5	4	2	7	4	8

TABLE I (continued)

Plasma Glucagon
(pmol/l)

Case No.	0'	10'	30'	60'	90'
1	12	12	8	13	17
3	13	7	11	8	5
6	30	30	27	27	28
7	33	32	22	30	40
9	23	48	35	39	35
10	21	24	14	15	26
12	9	9	5	6	17
13	25	26	23	21	27
14	18	20	23	17	16
15	19	11	13	14	10

TABLE I (continued)

Plasma NEFA
(μeq/l)

Case No.	0'	20'	40'	60'
1	862	506	372	353
2	610	338	269	325
3	732	541	405	321
4	-	-	-	-
5	774	424	330	378
6	830	464	328	370
7	584	458	279	298
8	597	328	240	288
9	717	410	280	326
10	732	360	252	313
11	630	354	269	239
12	696	377	312	360
13	620	413	212	340
14	762	310	190	260
15	544	260	164	330
16	588	369	232	211

APPENDIX (C)

TABLE I

Energy supplement during the 2nd period of the study.

SPECIAL MOUSSE

a) Recipe (10.5 oz Portion):

30g skim milk powder

133 mls water

1 level teaspoon Custard Powder

150 mls sunflower seed oil

sugar to taste

flavouring and colouring.

b) Analysis per 3½ oz portion:

Calories	514
Fluid	46 ml
Protein	3.3g
Fat	50g
Carbohydrate	9.5g
Sodium	2.5 mEq
Potassium	3.45 mEq
Calcium	121 mg
Phosphate	100 mg

Table 2. Mean daily food intake for each patient during 1st and 2nd period of study. 2nd period values are in brackets.

Case No.	Total energy (Kj)	Protein (g)	Fat (g)	Carbohydrate (g)	Carbohydrate (% of energy)	P/S ratio
3	9925 (13618)	80 (101)	149 (213)	173 (227)	29.3 (27.5)	0.19 (1.06)
4	10574 (17707)	55 (88)	147 (254)	243 (394)	38.5 (37.4)	0.15 (0.81)
5	9496 (9480)	67 (48)	126 (86)	214 (312)	37.9 (57.1)	0.11 (0.12)
9	10240 (8669)	55 (47)	120 (101)	283 (246)	46.6 (47.6)	0.14 (0.88)
10	4614 (4760)	36 (28)	52 (40)	120 (172)	44 (61)	0.16 (0.14)
12	13874 (12194)	110 (81)	160 (158)	354 (286)	43 (39.5)	0.19 (0.66)
13	8163 (8063)	51 (37)	103 (84)	201 (241)	41.3 (52.3)	0.11 (1.53)
14	8856 (5256)	29 (20)	79 (35)	316 (201)	60 (68.6)	0.08 (0.10)
16	10455 (9856)	55 (54)	181 (112)	158 (266)	25.4 (46.8)	0.09 (0.43)

APPENDIX (D)

Papers by the author based on the research described in this Thesis.

A. Published:

1. Hyperlipidaemia in children on regular haemodialysis.
   Arch. Dis. Child., 52, 932, 1977.

2. Hormonal and metabolic responses to intravenous glucose in children on regular haemodialysis.
   Am. J. Clin. Nutr., 31, 1865, 1978.

3. Plasma amino acids in children and adolescents on haemodialysis.
   Kidney International, 10, 471, 1976.

B. In press or submitted for publication:

1. Nutritional therapy in uraemic children.
   Am. J. Clin. Nutr. 1979 (in press).

2. Body composition of children on regular haemodialysis.
   Acta Paed. Scand., 1979 (submitted).

Archives of Disease in Childhood, 1977, **52**, 932–936

Hyperlipidaemia in children on regular haemodialysis

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From Evelina Children's Department, Guy's Hospital, London

SUMMARY Fasting plasma concentrations of triglycerides (TG), cholesterol, immunoreactive insulin (IRI), and blood glucose were raised in 16 children with chronic renal failure on regular haemodialysis compared with 18 healthy children. In the patients plasma IRI correlated positively with plasma TG, while blood glucose did not correlate with IRI or lipid concentrations. Dietary intake, expressed as percentage of recommended intake for height-age, did not correlate with plasma lipids, but there was a positive correlation between plasma TG and the proportion of calories derived from carbohydrate. The children were not malnourished as evidenced by normal plasma albumin and transferrin concentrations.

The mechanism of the hyperlipidaemia is unclear but it may be related to the glucose intolerance with hyperinsulinaemia which is found in uraemia. In view of the risk of premature atherosclerosis, plasma lipid concentrations should be monitored in children with chronic renal failure and attempts made to ameliorate hyperlipidaemia with appropriate dietary manipulations.

Hyperlipidaemia is a frequent finding in uraemic adults (Bagdade *et al.*, 1968; Gutman *et al.*, 1973; Ibels *et al.*, 1975). Many children with chronic renal failure are now being treated by regular haemodialysis and are often encouraged to take carbohydrate and fat supplements in their diet in an attempt to improve growth. An assessment of plasma lipids, particularly in relation to diet, therefore becomes important for rational management of these children.

We report plasma concentrations of triglycerides (TG) and cholesterol in children on regular haemodialysis with particular reference to diet, nutritional status, plasma concentrations of immunoreactive insulin (IRI), and blood glucose levels.

Patients

Sixteen children, 9 boys and 7 girls, with a mean age of 14 years (range 11–17 years) were studied. All patients were stable at the time of investigation and had been on dialysis for more than 6 months (range 7 months–6 years). None had the nephrotic syndrome, clinical or biochemical evidence of diabetes mellitus, or liver disease, or a known family history of hyperlipoproteinaemia. All the children were dialysed overnight at home for 30 hours per week in three sessions, 13 using Meltec Multipoint dialysers, 2 using Watson–Marlow Kiil type dialysers, and 1 using a Travenol Ultra-Flow dialyser. The dialysate had a dextrose concentration of 200 mg/100 ml. Apart from strict fluid and some sodium and potassium restriction, they ate a free diet with varying amounts of energy supplements based on a glucose polymer (Caloreen, Milner Scientific and Medical Research Co., Liverpool) and added double-cream (4 kcals/ml; 17 kJ/ml). Control values were obtained from 18 healthy British children in fasting state, 12 boys and 6 girls with a mean age of 12·3 years (range 10–17) requiring venepuncture for other reasons while inpatients for minor surgery. None had renal disease or systemic illness. They took a normal diet but intakes were not formally assessed. Consent was obtained in writing from the children's parents after full explanation of the procedures to both parents and children and the study was approved by the Medical School Ethical Committee.

Received 25 April 1977

Methods

Clinical. All patients were studied as outpatients at least 28 hours after dialysis and after an overnight fast of more than 12 hours. Blood samples were taken without stasis for the estimation of plasma concentrations of TG, cholesterol, IRI, albumin and transferrin, and blood glucose. The blood was immediately separated and plasma for IRI measurement was frozen at −20°C until assayed. Samples for plasma lipids were kept at 4°C and estimations and

;erum lipoprotein electrophoresis were carried out on he same day. Diet was assessed by prospective 3-day veighed records. All items of food and fluids taken by the child on three successive days, including one lialysis day, each month were recorded. Nutrient ntakes were calculated from the diet records using tandard tables (McCance and Widdowson, 1969). For this investigation the intake record closest to the ime of study was used and was within 3 weeks of the blood specimens being obtained. The dietary intakes vere expressed as a percentage of the recommended ntake (% RI) for the height-age of the child Department of Health and Social Security, 1969).

nalytical. Plasma TG was measured by the semi-utomated fluorimetric method of Cramp and Rob-rtson (1968) and plasma cholesterol by the auto-nated method of Levine and Zak (1964). The ouble-antibody immunochemical technique was sed to measure IRI (Morgan and Lazarow, 1963). lasma transferrin was determined by a single radial mmunodiffusion using Immuno-Plate III (Hyland). lasma albumin was measured by autoanalyser romocresol dye-binding method, Technicon). ipoprotein electrophoresis was performed on tarose gel. Blood glucose was measured by auto-nalyser (ferricyanide method, Technicon).

Statistical. Student's *t* test was used to compare plasma lipids, IRI, albumin, transferrin, and glucose in patients and controls. The relationship between plasma lipids and nutrition, IRI and TG, were analysed by least squares linear regression.

Results

Clinical data of the patients are given in Table 1. Table 2 gives the plasma lipids, IRI, albumin, and transferrin concentrations and blood glucose levels in the patients and controls. There were significant increases in both the plasma TG and plasma cholesterol concentrations in the patients compared with controls. No relationship was found between plasma lipid levels and age or sex of the patients or their ideal body weight for height-age. No patient had detectable chylomicrons or a broad beta-band on lipoprotein electrophoresis. Type IV hyperlipoproteinaemia was the predominant finding, occurring in 10 of the children. Four had type IIb and 2 had normal patterns. There was a significant increase in basal IRI and fasting blood glucose in the patients compared with controls. The fasting plasma TG was positively correlated with basal IRI (Fig. 1), but not with fasting blood glucose. IRI and fasting blood glucose were not correlated. Plasma albumin

ible 1 *Clinical data of patients*

se	Sex	Renal disease	Age (yrs)	Height (cm)	Height centile	Weight (kg)	Duration on dialysis (yrs)
	F	Reflux nephropathy	17·8	151·1	<3rd	40	3·5
	F	,, ,,	12·4	152·1	75th	45	1·0
	M	,, ,,	12·0	132·1	<3rd	28·7	5·5
	F	,, ,,	12·8	127·4	<3rd	23	3·0
	F	,, ,,	11·3	128·1	3rd	24·5	0·58
	M	Focal glomerulosclerosis	16·0	143·6	<3rd	56·9	2·0
	M	,, ,,	12·3	141·4	25th	29	1·0
	F	,, ,,	14·1	139·9	<3rd	41	3·0
	M	Chronic glomerulonephritis	15·0	160·1	50th	45	1·5
	M	,, ,,	14·6	139·1	<3rd	35·5	6·5
	F	Juvenile nephronophthisis	12·8	142·1	10th	30·4	2·5
	F	,, ,,	14·9	140·1	<3rd	49·2	0·75
	M	Single dysplastic kidney	16·0	148·0	<3rd	40	1·5
	M	Obstructive uropathy	14·8	136·6	<3rd	29·5	1·5
	M	Cystinosis	14·5	127·4	<3rd	31	1·0
	M	,,	11·7	129·3	<3rd	32	2·0

ble 2 *Biochemical results in patients and controls (mean±1SD)*

	n	TG (mmol/l)	Cholesterol (mmol/l)	IRI (mU/l)	Blood glucose (mmol/l)	Plasma albumin (g/l)	Plasma transferrin (g/l)
nts	16	1·974±0·92	6·27±1·27	20·7±8·7	5·07±0·38	42±4·8	2·41±0·91
trols	18	0·838±0·25	4·49±0·82	8·4±5·2	4·28±0·54	43±1·9	2·46±0·52
ificance of difference, P		<0·001	<0·001	<0·001	<0·001	NS	NS

triglycerides; IRI = immunoreactive insulin.
ersion: SI to traditional units—Triglyceride: 1 mmol/l≈80·5 mg/100 ml. Cholesterol: 1 mmol/l≈38·6 mg/100 ml. Blood glucose: nol/l≈18 mg/100 ml.

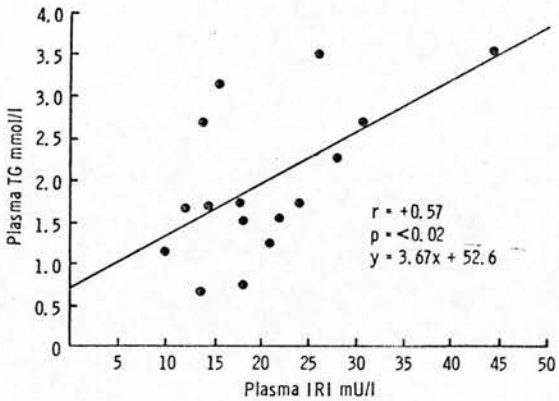


Fig. 1 *Plasma triglyceride (TG) concentrations related to plasma immunoreactive insulin (IRI) levels in 16 children on regular haemodialysis.*

and transferrin concentrations, as indices of nutritional state, were similar in patients and controls and did not correlate with plasma lipid levels.

Effect of diet. The dietary intakes of total energy, carbohydrates, total fat and proteins expressed as % RI for the height-age are presented in Table 3. The relationship between the dietary intakes and plasma TG and cholesterol concentrations in the patients is shown in Table 4. There was a tendency for those children with lower intakes of total energy, fats, and proteins to have higher TG levels but TG and carbohydrate intake were poorly correlated. However, when expressed as a percentage of the total energy intake, there was significant positive correlation between plasma TG concentration and percentage of calories derived from carbohydrate (Fig. 2). No correlation between plasma cholesterol concentration and food intakes could be shown.

Discussion

Raised plasma triglycerides have been commonly reported in adults on regular haemodialysis (Bagdade *et al.*, 1968; Gutman *et al.*, 1973) and recently Pennisi *et al.* (1976) and Broyer *et al.* (1976) reported raised TG levels in children on dialysis. Our group of children showed a similar phenomenon. Type IV hyperlipoproteinaemia is commonly found and the majority of our patients exhibited this pattern. Raised TG levels in uraemia are considered to be due to both increased hepatic TG production (Bagdade *et al.*, 1968; Cramp *et al.*, 1976) and decreased TG clearance from plasma (Cattran *et al.*, 1976; Murase *et al.*, 1975). Our finding of high basal plasma insulin levels in the face of raised fasting blood glucose is indicative of glucose intolerance. The positive

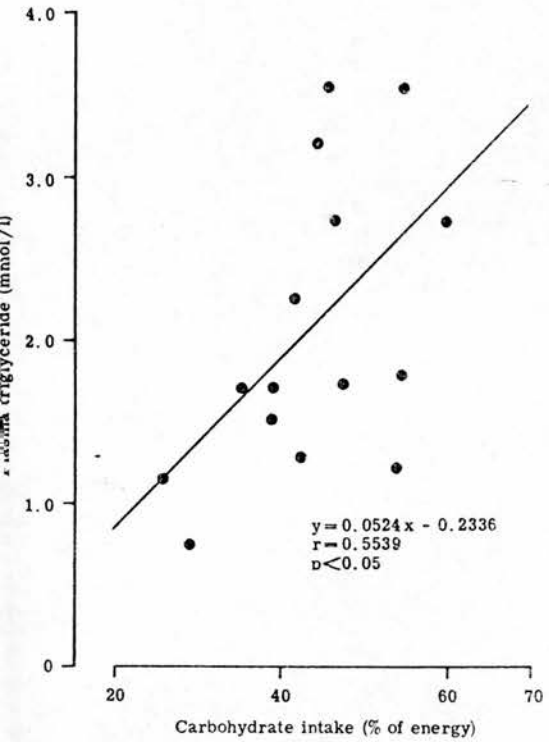
Table 3 *Diets of children on dialysis*

Case no.	*Energy*		*Protein*		*Carbohydrate*		*Fat*	
	kJ/day	*%RI*	*g/day*	*%RI*	*g/day*	*%RI*	*g/day*	*%RI*
1	9 520	99	60	103	303	101	97	114
2	12 127	126	132	228	244	82	156	184
3	14 102	134	84	133	378	109	171	184
4	10 964	125	56	106	321	118	128	162
5	15 911	181	118	183	396	132	198	251
6	11 698	112	79	125	327	101	135	145
7	10 983	105	77	122	246	102	150	161
8	12 493	130	64	110	389	130	215	253
9	12 001	102	66	88	265	80	173	166
10	4 886	47	46	73	136	42	51	55
11	—	—	—	—	—	—	—	—
12	10 306	107	68	117	297	109	116	136
13	18 684	159	128	70	389	107	268	258
14	6 415	61	46	73	183	56	71	76
15	9 175	104	55	104	237	87	116	147
16	8 010	91	18	34	302	111	76	96

%RI = percentage of recommended intake.
Conversion. SI to traditional units—Energy: 1 kJ≈0·239 kcal.

Table 4 *Relationship between diet (as % of RI for height-age) and plasma lipid concentrations*

	Total energy		*Fat*		*Carbohydrate*		*Protein*	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Triglycerides	−0·37	NS	−0·46	<0·1	−0·12	NS	−0·38	NS
Cholesterol	−0·13	NS	−0·06	NS	−0·18	NS	+0·13	NS



. 2 Plasma triglyceride concentrations related to bohydrate intake (% of total energy intake) in 15 ldren on regular haemodialysis.

relation shown between plasma insulin and TG ıcentration is similar to that found in uraemic ılts (Bagdade, 1970) and it is possible that hyper-ılinaemia might stimulate an increased hepatic production, as suggested for nonuraemic subs (Reaven *et al.*, 1967; Olefsky *et al.*, 1974).

he role of diet in the pathogenesis of hyper-lyceridaemia in uraemia is not clear. No correla- existed between total energy intake and TG :entration, though higher total energy and pro- intakes tended to be associated with lower levels. TG levels reported here are about 65% lower those reported by Broyer *et al.* (1976) in lren on dialysis, and energy and protein intakes ur children were considerably greater than in study. However, other differences exist in the ıgement of these children, for instance our ren dialyse for about 30 hours a week compared 16 hours a week. The proportion of total energy e derived from carbohydrate correlated signi-ly with TG concentrations. Both Pennisi *et al.*) and Broyer *et al.* (1976) showed a similar onship between dietary carbohydrate and ıa TG concentration. More recently, Sanfelippo *et al.* (1977) reported a reduction in plasma TG levels by feeding diet low in carbohydrate and high in polyunsaturated fat. Unfortunately it is not known from their study which of the two variables in the diet altered the TG levels.

Plasma cholesterol concentrations are usually found to be normal in uraemic adults whereas our patients showed considerable increases both compared with controls and with the children reported by Broyer *et al.* (1976), though 6 of 17 children in the latter study also showed significantly increased levels. We were unable to show any relationship between diet and plasma cholesterol, though Broyer *et al.* suggested a relationship with lipid intake. As our patients were taking energy supplements based on food high in saturated fats, it is possible that manipulation of the diet by increasing the polyunsaturated fat intake might lead to a fall in plasma cholesterol, and we are attempting this at present.

The finding of hyperlipidaemia in children on regular haemodialysis awaiting transplantation is disturbing in view of the relationship between hyperlipidaemia and arterial disease shown in prospective studies of normal populations (Kannel *et al.*, 1971; Carlson and Böttiger, 1972) and the increased incidence of premature atherosclerosis and cardiovascular disease reported in adult patients on long-term haemodialysis (Lowrie *et al.*, 1973; Lindner *et al.*, 1974; Bagdade, 1975). Pennisi *et al.* (1976) studied the morphology of coronary arteries in children who died while on regular haemodialysis for renal failure. In comparison with children dying of other diseases at a similar age, there were changes suggestive of fat deposition in the arterial walls.

While the exact mechanism of the hyperlipidaemia remains obscure, it obviously is important to measure TG and cholesterol concentration both in children on haemodialysis and in children with chronic renal insufficiency who have not yet required dialysis or transplantation. If raised levels are found, attempts can be made to lower them by dietary manipulation or increased dialysis. The need to ensure adequate nutrition in children in renal failure has been emphasized (Chantler and Holliday, 1973). Further studies of the effect of various dietary constituents on plasma lipids are required, but in the meantime it seems reasonable to try to ensure an adequate intake of total energy and protein. Regular diet surveillance is important and for those children with raised lipid levels the proportion of carbohydrate and saturated fat in the diet may need to be controlled.

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Hormonal and metabolic responses to intravenous glucose in children on regular hemodialysis[1, 2]

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ABSTRACT Blood glucose, plasma nonesterified fatty acids, amino acids, immunoreactive insulin, growth hormone, and immunoreactive glucagon responses to intravenous glucose were determined in 16 children on regular hemodialysis for chronic renal failure and nine healthy children. In the patients the fractional disappearance rate of glucose was significantly reduced, basal immunoreactive insulin was significantly raised, and while the early immunoreactive insulin response to glucose was similar in patients and controls, the late response was increased. Basal growth hormone was elevated in the patients and rose paradoxically following glucose. Fasting immunoreactive glucagon was significantly higher in the patients and was not suppressed by glucose. Plasma nonesterified fatty acid levels were lower in the patients and fell more markedly after glucose. Alanine levels, which were significantly raised in those with poor glucose tolerance, fell to normal after glucose and did not vary in those with more normal glucose tolerance. It is speculated that the metabolic and hormonal alterations may be interrelated and result from failure of normal glucose utilization. *Am. J. Clin. Nutr.* 31: 1865–1869, 1978.

Most children with chronic renal failure fail to grow normally and are, therefore, insufficiently anabolic. An adequate supply of dietary energy and protein may improve growth rate but does not ensure it (1). The increased catabolic responses to stress in uremic rats (2) and the possibility of increased energy and protein requirements for anabolism in acutely uremic children (3) suggest the possibility of defective energy utilization resulting in altered protein metabolism (4).

The responses of the major fuel substrates, glucose (BG), nonesterified fatty acids (NEFA) and amino acids (AA) and the more important metabolic hormones, insulin, growth hormone (GH), and glucagon (IRG) following intravenous glucose, were studied in a group of children on regular hemodialysis for renal failure in an assessment of hormone-fuel interrelationships.

Materials and methods

Sixteen children, nine boys and seven girls, with mean age of 13.9 years were studied. All were stable at the time of investigation and had been on dialysis for at least 6 months (range 0.58 to 5.6 years). None had the nephrotic syndrome, clinical or biochemical evidence of diabetes mellitus, or liver disease. All were dialyzed with a glucose concentration of 200 mg/dl. Apart from fluid, sodium, and potassium restriction, they ate a free diet with varying amounts of energy supplements based on a glucose polymer (Caloreen; Milner Scientific and Medical Research Co., Liverpool, England), and double cream (4 kcal/ml). Controls were nine healthy children, five boys and four girls, with a mean age of 12.5 years. Studies were performed after an overnight fast of at least 14 hr, and at least 28 hr postdialysis.

Glucose, 0.5 g/kg of a 50% solution was injected intravenously over a period of 3 min and blood samples were taken at 0, 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min. BG was measured by autoanalyser (Ferricyanide method, Technicon). Radioimmunoassay techniques were used for the determination of insulin (5), GH (6), and IRG (7). Plasma NEFA was measured by a semiautomated fluorimetric method (8). The plasma amino acids methodology and results have been reported (4). Glucose disappearance rate (K) was calculated by the method of least squares regression after logarithmic transformation. The early insulin response (0 to 10 min)

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was calculated by subtracting the fasting value from the means of the 5 and 10 min values. The later insulin response was calculated as the means of absolute values between 20 and 60 min following glucose injection.

Student's t test was used to compare results in patients and controls. The significance of changes in response to glucose was assessed by paired t tests.

Results

Clinical data on the patients have been published (4). Fasting BG was significantly raised in the patients ($P < 0.001$) and the rate of glucose disappearance (K value) significantly reduced (Fig. 1 and Table 1). Fasting insulin levels were significantly elevated in the patients ($P < 0.01$). Following glucose injection, the early insulin response (0 to 10 min) was similar in patients and controls. The late response (20 to 60 min) was significantly raised in the patients (Fig. 2 and Table 1).

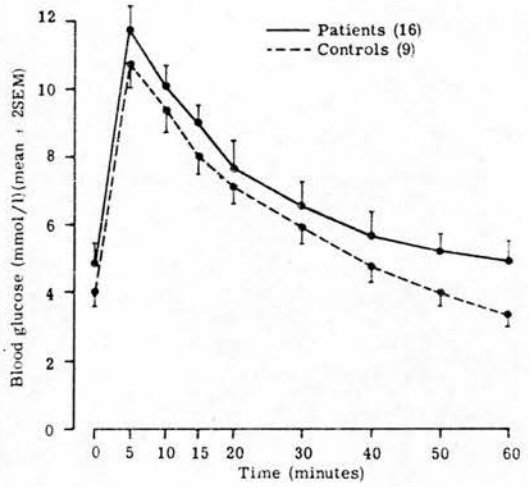

FIG. 1. Mean ± 2 SEM of blood glucose values during IVGTT in patients and controls.

Fasting GH levels were significantly raised in the patients ($P < 0.02$) and rose paradoxically following intravenous glucose (Fig. 3). Basal plasma IRG levels were significantly increased in the patients ($P < 0.005$). After glucose injection IRG fell significantly in the controls, but remained elevated throughout the test in the patients (Fig. 4). Fasting NEFA were significantly reduced in patients, and fell more markedly and for longer in response to intravenous glucose; the levels being significantly lower 40 and 60 min after the glucose injection (Fig. 5). Reductions in AA compared with normal, were found in valine, leucine, isoleucine, lysine, histidine, tyrosine, and serine; and increases in glycine, citrulline, proline, and 1- and 3-methyl histidine. Acute reductions in AA concentrations occurred in response to intravenous glucose similar to those reported in normal adults (9), but plasma alanine, which was significantly raised in those with poor glucose tolerance, fell to normal, and did not vary in those with more normal glucose tolerance.

Discussion

Carbohydrate intolerance is well documented in adult uremic patients and peripheral insulin resistance seems to be the chief cause (10). The glucose disappearance rate (K) was reduced in the majority of our patients. The basal differences in BG concentration could be partly due to the nonspecific method of measurement used. However, interfering substances do not change during the glucose tolerance test and so the dynamic changes of BG during the test are valid (11).

The normal early insulin response found in this study excludes a defect in pancreatic

TABLE 1
Mean ± 1 SD of plasma insulin (munits/liter) in response to intravenous glucose in children on hemodialysis and controls

	Fasting	Early response (0–10 min)	Late response (20–60 min)	K value for glucose
Patients (16)	16.7 ± 7.7	33.2 ± 13	25.7 ± 12.7	1.09 ± 0.28
Controls (9)	8.2 ± 3.7	24.6 ± 11.2	11.8 ± 3.6	1.84 ± 0.30
Significance of difference (P)	<0.01	NS[a]	<0.005	<0.001

[a] Not significant.

insulin secretion as a cause of the glucose intolerance. However, the raised BG levels, in spite of elevated plasma insulin is indicative of peripheral resistance to insulin. The degree of glucose intolerance did not correlate with plasma GH, IRG, or NEFA and the precise nature of this resistance remains obscure. It is of interest that fasting plasma NEFA levels were significantly lower in the patients and fell more markedly following glucose. This suggests a decrease in lipolysis and/or increased reesterification of NEFA in the uremic state due to high insulin levels, and that adipose tissue is not resistant to the action of insulin.

Elevated plasma GH has been reported in

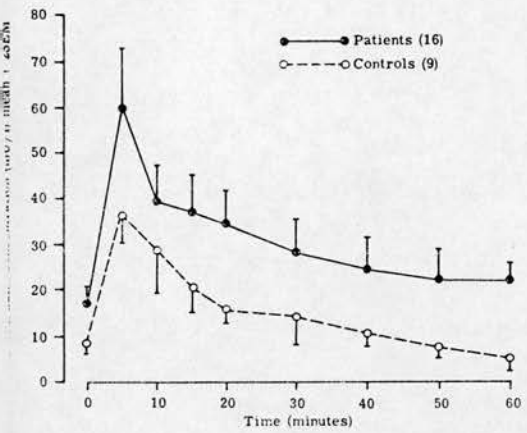

FIG. 2. Mean ± 2 SEM of plasma immunoreactive insulin, fasting and in response to intravenous glucose in patients and controls.

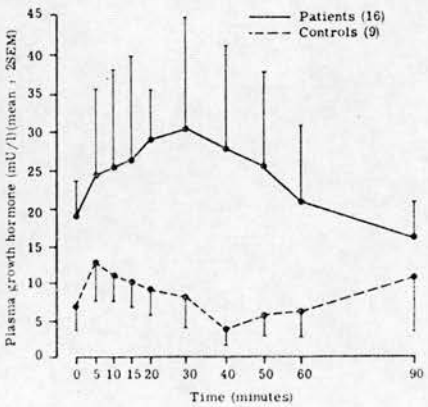

FIG. 3. Mean ± 2 SEM of plasma growth hormone concentrations, fasting and in response to intravenous glucose in patients and controls.

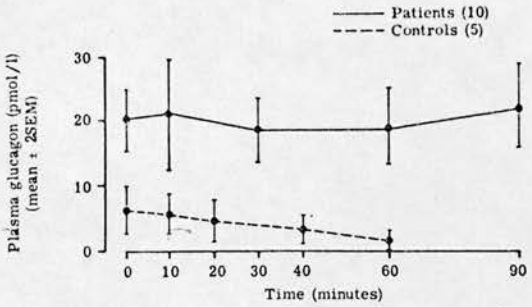

FIG. 4. Mean ± 2 SEM of plasma immunoreactive glucagon, fasting and in response to intravenous glucose in patients and controls.

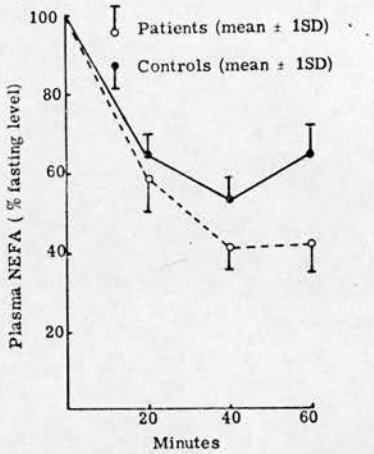

FIG. 5. Percentage fall (Mean ± 1 SD) of plasma nonesterified fatty acids following intravenous glucose.

adults with chronic renal failure and is due to increased secretion, as shown by the prompt reduction in levels following somatostatin administration (12, 13). Wright et al. (12) suggested that it could be due to protein malnutrition but Davidson et al. (14) found no correlation between GH concentration and dietary protein; nor could we demonstrate any correlation between plasma GH concentration and either plasma albumin or transferrin as indices of nutritional status, or with dietary protein. It is possible that the intracellular energy supply is deranged in uremia and that GH secretion is increased in an attempt to mobilize fat for energy and preserve body protein. The rise in GH after glucose is interesting. Is the normal GH response to glucose somehow ablated in uremia and the falling plasma NEFA levels stimulating its secretion? There is evidence that

plasma NEFA have a regulatory effect on GH secretion (15).

If both endogenous fat mobilization and NEFA oxidation are decreased in uremia (16, 17) in the face of a failure of glucose utilization, this might lead to increased utilization of AA as energy substrate. We reported these children to have a significant reduction in the branched-chain AA (4) which are known to be oxidized in muscle (18). Alanine, the major gluconeogenic substrate, was found to be high in those patients with poor glucose tolerance. This might be an indication of its increased peripheral release as essential amino acids were used up for energy and broken down for gluconeogenesis.

Hyperglucagonemia is well recognized in uremic adults and is due probably to increased secretion as well as decreased degradation of glucagon (19, 20). Although nonsuppressible in our study, Kuku et al. (21), in demonstrating that it was a heterogeneous molecule, showed that the fragment which corresponds to standard glucagon did fall in concentration after glucose, though not to normal levels. Sherwin et al. (20) also suggest that tissue sensitivity to glucagon is increased in uremia. It is not known why glucagon secretion is increased in uremia but it may be stimulated by the retention of nitrogenous compounds (22) or a response to glucopenia induced by failure of normal glucose transport or metabolism.

Increased protein breakdown, alanine release, and gluconeogenesis (23) are in keeping with hyperglucagonemia. Felig et al. (24) have shown that in fasted man, hepatic gluconeogenesis is limited by substrate availability. In prolonged starvation adaptive mechanisms which conserve protein come into play and substrate supply for gluconeogenesis is decreased. There is increased utilization of fat for energy. Such an adaptation may be denied to the uremic patient.

The use of intravenous glucose as a tool to investigate hormonal and metabolic interrelationships has revealed different responses in children with chronic renal failure compared with healthy children. On the basis of these findings we speculate that the prime defect is a failure of normal glucose uptake by cells and decreased fat availability for energy production leading to increased protein catabolism and in the case of children, failure of growth.

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Plasma amino acids in children and adolescents on hemodialysis

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Plasma amino acids in children and adolescents on hemodialysis. Fasting plasma amino acid concentrations were measured in 16 children on regular hemodialysis for renal failure. Reductions compared to normal were found in valine, leucine, isoleucine, lysine, histidine, tyrosine, and serine; and increases were found in glycine, citrulline, proline, and 1- and 3-methylhistidine. Acute reductions in amino acid concentrations occurred in response to i.v. glucose, similar to those reported in normal adults, but plasma alanine, which was raised only in those with poor glucose tolerance, fell to normal and did not vary in those with normal glucose tolerance. No correlations were found with growth, but the plasma glycine concentration was highest in those patients with poorest energy intakes. Plasma alanine concentrations correlated with raised triglyceride concentrations. It is suggested that many of the abnormalities are due to the excessive utilization of protein for energy because of impaired availability of conventional energy sources in uremia.

Acides aminés du plasma chez les enfants et adolescents en hémodialyse. Les concentrations des acides aminés du plasma ont été mesurées à jeun chez 16 enfants en hémodialyse iterative pour insuffisance rénale. Par comparaison avec des sujets normaux, des diminutions ont été constatées pour valine, leucine, isoleucine, lysine, histidine, tyrosine et sérine et des augmentations pour glycine, citrulline, proline et 1- et 3–méthylhistidine. Des dimunutions aigues des concentrations d'acides aminés ont été observées en réponse à l'administration intraveineuse de glucose, semblable à celles rapportées chez les adultes normaux, à l'exception de l'alanine dont la concentration n' a pas été modifiée chez les sujets ayant une tolérance au glucose normale et a été abaissée à des cours normales, à partir d'une valeur élevée, chez les sujets ayant une tolérance au glucose diminuée. Aucune corrélation n' a été observée avec la croissance mais la concentration plasmatique de glycine est la plus élevée chez les malades dont l'apport énergétique est le plus faible. Les concentrations plas matiques d' alanine sont élées avec l'augmentation de la concentration des triglycérides. est suggéré que beaucoup de ces anomalies sont liées à lisation excessive des protéines comme source d'énergie en on de la diminution des sources d'énergie conventionnelles an s de l'urémie.

Plasma amino acid concentrations are abnormal in uremic adults even after treatment by hemodialysis [1, 2, 3]. The concentrations of some essential amino acids are frequently reduced and those of nonessential amino acids are normal or increased. Certain changes, such as the reduced ratio of tyrosine to phenylalanine [4], decreased valine/glycine ratio [5], and increased citrulline concentrations [6] are characteristic, while others such as alterations of histidine and phenylalanine concentrations vary from study to study. Some of these changes are also seen in energy-protein malnutrition and it has been difficult to distinguish whether changes in uremia reflect a specific metabolic disturbance, or are secondary to malnutrition.

There is little published information on the changes in children. We have measured plasma amino acid concentrations in a group of children on regular dialysis and related them to growth, nutrition, and other biochemical parameters.

Methods

Subjects. Sixteen children, 9 boys and 7 girls with a mean age of 14 years (11 to 17 years) who had been on regular hemodialysis in the home for more than six months (seven months to six and one-half years), were studied (Table 1). None had any systemic disease and all were stable at the time of investigation. They dialyzed for ten hours overnight on three nights of each week, 13 using Meltec Multipoint dialysers, two using Watson-Marlow Kiil type dialyzers and one using a Travenol Ultra-Flo dialyzer. The dialysates had a glucose concentration of 200 mg/100 ml. Normal meals were eaten and dietary restrictions were confined to sodium and potassium, where necessary, and fluid intake was limited to approximately 300 ml per day. Each child also took a variable amount of a highly concentrated energy mixture based on double cream and a glucose polymer (Calo-reen®, Milner Scientific and Medical Research Company, Liverpool, England). Control values were obtained from children having venepuncture for other reasons while inpatients for minor surgery. None had

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Table 1. Clinical data of children on hemodialysis

Renal disease	Age, *yrs*	Bone age	Height, *cm*	Height centile		Height velocity, *cm/yr*		Duration on dialysis, *yr*
				chron. age	bone age	actual	expected for bone age	
Reflux nephropathy	17.8	15.0	150.1	<3rd	3rd-10th	1.0	1.2	3.5
Reflux nephropathy	12.4	13.5	152.3	75th	25th-50th	6.5	4.5	1.0
Reflux nephropathy	12.0	8.0	132.1	<3rd	75th-90th	4.2	5.5	5.5
Reflux nephropathy	12.8	8.5	127.4	<3rd	50th	3.4	5.4	3.0
Reflux nephropathy	11.3	11.0	128.1	3rd	3rd	4.1	6.1	0.58
Focal glomerulosclerosis	16.0	14.0	143.6	<3rd	<3rd	1.1	7.3	2.0
Focal glomerulosclerosis	12.3	12.5	141.4	25th	25th	1.9	5.5	1.0
Focal glomerulosclerosis	14.1	8.0	139.9	<3rd	>97th	2.6	5.5	3.0
Chronic glomerulonephritis	15.0	15.0	160.1	50th	50th	5.8	5.8	1.5
Chronic glomerulonephritis	14.6	10.0	139.1	<3rd	50th-75th	6.2	5.1	6.5
Juvenile nephronophthisis	12.8	12.0	142.1	10th	25th	1.6	5.0	2.5
Juvenile nephronophthisis	14.9	15.0	140.1	<3rd	<3rd	2.6	1.1	0.75
Single dysplastic kidney	16.0	—	148.0	<3rd	—	—	—	1.5
Obstructive uropathy	14.8	10.0	136.6	<3rd	50th	—	—	1.5
Cystinosis	14.5	11.5	127.4	<3rd	<3rd	5.5	4.9	1.0
Cystinosis	11.7	11.0	129.3	<3rd	3rd	2.3	5.0	2.0

disease or systemic disturbance, and no dietary nation was available.

e study was approved by the Medical School al Committee and consent obtained in writing the children's parents after full explanation to parents and children.

thods. After an overnight fast of at least 14 hr on, 24 to 48 hr after finishing a dialysis, blood es were collected without stasis in the resting rom the venous side of arteriovenous fistulae eparinized tubes. Thirteen of the children also v. glucose tolerance tests (IVGTT = 0.5 g of ucose per kg of body weight) and blood sam- r amino acid analysis were obtained after fast- d at one hour after glucose injection. The s were separated promptly, deproteinized with ic acid, to which norleucine had been added as l standard and stored at −20°C prior to analy- nino acids were measured by ion-exchange ıtography on an automatic amino acid ana- .KB 3201). A three sodium buffer step system ed with a constant operating temperature of Aliquots of plasma were also analyzed for rides by a semiautomated method [7], for cho- by the automated method of Levine and Zak for nonesterified fatty acids (NEFA) by a omated fluorimetric method [9]. Blood glu- ıs measured by autoanalyzer (ferricyanide Technicon), plasma albumin by autoanaly- mocresol dye-binding method, Technicon), ırea by autoanalyzer (diacetylmonoxine reac- ısma creatinine by autoanalyzer (Technicon, 11b), plasma transferrin by single radial im- usion, and plasma insulin by double an- dioimmunoassay [10].

Linear growth was measured according to the method of Tanner, Whitehouse, and Takaishi [11], and bone age was assessed by independent observers using the method of Greulich and Pyle [12].

Food intakes were assessed by three-day prospective weighed recordings done at home, usually on Friday, Saturday, and Sunday and including one dialysis day (Table 2). These are performed regularly and the intakes used for this study were all recorded within three weeks of the blood samples being obtained. The dietary intakes were expressed as a percentage of the recommended dietary allowance [13], according to the height age of the child.

Statistical methods. Student's *t* test was used to compare amino acid concentrations in patients and controls. The relationships between amino acids and growth, nutrition, and other biochemical parameters were analyzed by least squares linear regression. The significance of changes in amino acid concentrations in response to glucose was assessed by paired *t* tests.

Results

The means and standard deviations of plasma amino acid concentrations in patients and controls are shown in Table 3. Values are not shown for threonine, because of its poor separation from glutamine; tryptophan, because of its appreciable reduction by the use of picric acid as a deproteinizing agent [14]; and glutamine because of its cyclization to pyrrolidine carboxylic acid known to occur at the column temperature used [15].

There were significant reductions in concentrations of valine, leucine, isoleucine, lysine, tyrosine, serine, and histidine. Glycine, citrulline, proline, and 1- and 3-methylhistidine were significantly elevated, while

Table 2. Diets of children on dialysis

Patient No.	Energy		Protein		Carbohydrate		Fat	
	kjoules/day	%RDA[a]	g/day	%RDA[a]	g/day	%RDA[a]	g/day	%RDA[a]
1	9520	99	60	103	303	101	97	114
2	12127	126	132	228	244	82	156	184
3	14102	134	84	133	378	109	171	184
4	10964	125	56	106	321	118	128	162
5	15911	181	118	183	396	132	198	251
6	11698	112	79	125	327	101	135	145
7	10983	105	77	122	246	102	150	161
8	12493	130	64	110	389	130	215	253
9	12001	102	66	88	265	80	173	166
10	4886	47	46	73	136	42	51	55
11	—	—	—	—	—	—	—	—
12	10306	107	68	117	297	109	116	136
13	18684	159	128	70	389	107	268	258
14	6415	61	46	73	183	56	71	76
15	9175	104	55	104	237	87	116	147
16	8010	91	18	34	302	111	76	96

[a] RDA = Recommended Daily Allowance.

the remainder were within normal limits. Alanine was at the upper limit of normal. The ratio of tyrosine to phenylalanine was consistently reduced, mainly due to the reduction in tyrosine concentration. The valine/glycine ratio was also low because of the reduced valine concentration and raised glycine concentration.

Effect of diet and nutritional state. There was an inverse linear relationship between glycine concentration and fat and energy intakes (Table 4). The valine/glycine ratio showed a similar, though not as close a relationship, and the plasma valine concentration was not influenced by diet at all. However, there was a direct linear relationship between the plasma valine concentration and plasma albumin, significant at the 5% level. A direct linear relationship was shown between the plasma citrulline concentration and energy and protein intake (Table 4). Other amino acid concentrations did not correlate at the 5% level with albumin and there was no correlation between amino acid concentrations and transferrin and hemoglobin concentrations. The plasma albumin concentrations were all within the normal range (3.6 to 5.3 g/100 ml) and did not correlate with diet.

Amino acids and growth. Each amino acid was analyzed in relation to the degree of growth failure, growth velocity, and bone maturity and no significant associations were found.

Amino acids and lipids. Full results of plasma lipid determinations will be published elsewhere. Plasma

Table 3. Plasma amino acid concentrations in patients and controls

Amino acid	Patients			Controls			
	No.	Mean nmol/ml	SD	No.	Mean nmol/ml	SD	P
Valine	16	145	29	8	207	27	<0.001
Leucine	16	69	13	8	102	16	<0.001
Isoleucine	16	42	13	8	52	7	<0.05
Lysine	15	155	23	8	181	26	<0.05
Methionine	14	14	4	7	17	2	NS
Phenylalanine	16	39	9	8	40	5	NS
Histidine	16	70	11	8	86	19	<0.05
Alanine	16	380	150	8	275	49	NS
Glycine	16	405	113	8	224	30	<0.001
Citrulline	16	89	22	8	78	7	<0.001
Serine	15	83	21	7	163	27	<0.001
Tyrosine	16	23	6	8	40	8	<0.001
Cystine	15	56	15	2	35	3	NS
Ornithine	15	62	15	8	59	18	NS
Arginine	13	74	21	8	70	10	NS
Proline	15	231	105	7	133	37	<0.05
Tyrosine/phenylalanine	16	0.61	0.12	8	0.99	0.17	<0.001
Valine/glycine	16	0.39	0.14	8	0.93	0.19	<0.001

Table 4. Relationship between diet[a] and plasma amino acid concentrations

Amino acid	Protein		Fat		Carbohydrate		Energy	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Glycine	−0.41	NS	−0.60	<0.05	−0.25	NS	−0.55	<0.05
Valine	+0.19	NS	+0.14	NS	+0.14	NS	+0.22	NS
Valine/glycine	+0.34	NS	+0.56	<0.05	+0.19	NS	+0.55	<0.05
Citrulline	+0.52	<0.05	+0.48	0.1	+0.43	0.1	+0.54	<0.05

[a] As percentage of Recommended Daily Allowance for height and age.

triglyceride concentrations were significantly raised and correlated with plasma alanine ($r = +0.55$; $N = 16$; $P < 0.05$). Plasma cholesterol concentrations were also significantly raised and correlated inversely with plasma proline ($r = -0.57$; $N = 15$; $P < 0.05$) and plasma proline concentrations correlated directly with plasma nonesterified fatty acids (NEFA) ($r = +0.61$; $N = 15$; $P < 0.05$). The NEFA tended to be low, but not significantly so.

The effect of glucose. There was a significant mean reduction in the concentrations of valine, leucine and isoleucine, tyrosine, alanine, lysine, ornithine and serine 60 min after glucose injection (Table 5). Other amino acids also fell in concentration, but not significantly. These changes did not correlate with the peak, or the 60 min plasma insulin concentrations.

The concentration of alanine 60 min after glucose injection was expressed as a percentage of the fasting level and compared to the glucose disappearance rate (K) during the IVGTT. There was a close linear relationship between the percentage reduction and the K values (Fig. 1). The fasting concentrations of alanine correlated inversely with the K values for glucose ($r = -0.62$; $N = 13$; $P < 0.05$); thus, the worse the glucose tolerance, the higher the fasting concentration of alanine and the greater its fall after glucose. Those with better glucose tolerance had normal plasma alanine concentrations, which showed little or no change when glucose was injected. These findings did not apply to any of the other amino acids.

Amino acids and residual renal mass and uremia. Two patients were anephric and analysis of their results showed no significant difference in comparison to the remainder. No plasma amino acid concentration correlated with that for creatinine but the plasma urea concentration correlated inversely both with the plasma glycine ($r = -0.6031$; $P < 0.05$) and plasma alanine concentrations ($r = -0.6060$; $P < 0.05$).

Table 5. Plasma amino acid concentration during fasting and 60 min after glucose infusion

Amino acid	No. of patients	Plasma concentration, *nmol/ml*				
		During fasting		60 min after glucose infusion		
		mean	SEM	mean	SEM	*P*
Valine	13	164	10	146	9	<0.001
Leucine	13	73	5	62	4	<0.001
Isoleucine	13	44	3	37	3	<0.01
Lysine	11	159	10	148	9	<0.05
Methionine	9	11	2	9	1	NS
Phenylalanine	13	38	2	38	2	NS
Histidine	13	69	5	69	4	NS
Arginine	13	75	5	76	5	NS
Glycine	12	392	30	385	28	NS
Alanine	13	337	39	298	27	<0.05
Serine	13	84	6	75	4	<0.05
Cystine	11	60	6	58	6	NS
Ornithine	11	60	5	53	3	<0.05
Tyrosine	13	21	1	17	1	<0.01
Citrulline	13	92	4	84	4	NS

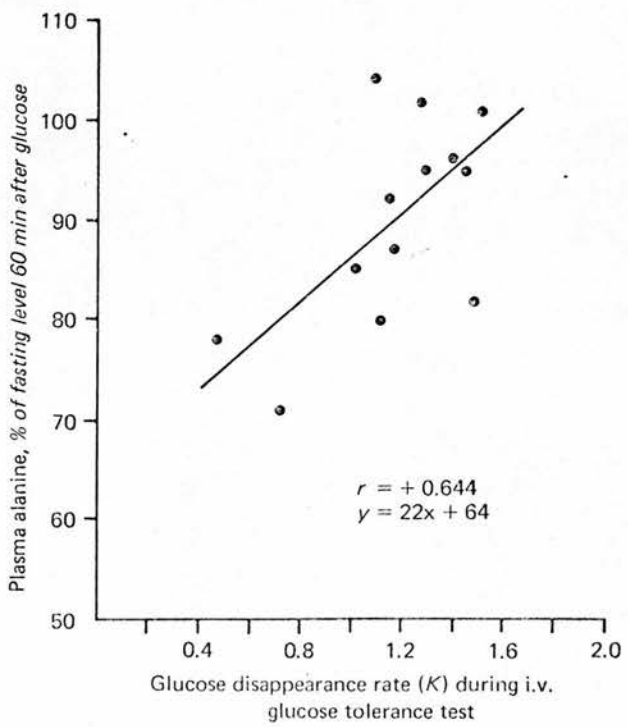

Fig. 1. *Relationship between the percentage of reduction in plasma alanine concentration 60 minutes after glucose injection and the K values for glucose during i.v. glucose tolerance tests.*

Discussion

These patients were at the upper age range of childhood for practical reasons; five were pubertal, four were in early puberty, and the remainder were prepubertal. Most had a retarded bone age (Table 1). While age does influence plasma amino acid concentrations, there is no evidence that significant changes occur over the age range we studied, and the controls were of a comparable age.

Comparison with similar reports on adults on hemodialysis shows how much interstudy variation exists. Direct quantitative comparisons are restricted by differences in methodology, such as sample preparation and analytical procedures. In most reports there is little information on dietary intakes and it is likely that considerable variation exists. The effect of heterogeneity of body composition is not known, nor are the effects of residual renal mass. Our data do not show significant differences between anephric patients and others but are too scanty in this respect to be helpful.

Reductions in the concentrations of the branched-chain amino acids—valine, leucine and isoleucine—and tyrosine and lysine are the changes most consistently found. McGale, Pickford, and Aber [17] have found glycine to be increased as we have, whereas Held et al [3] have reported a reduction not only in glycine, but also in alanine and phenylalanine. Phenylalanine has been most frequently reported to be normal in concentration in uremic patients, both on and off dialysis [1]. Histidine concentrations may be normal [2], or reduced [1], while 1- and 3-methylhistidine concentrations are typically increased [1, 17]. The ratio of essential to nonessential amino acids is reduced in uremia, but we have not analyzed this, because Kopple and Swendseid [5] have shown that this ratio is a function of protein intake, rather than uremia. In addition we have excluded some amino acids from analysis for technical reasons and this would affect the ratio. We suggest that the differences found from study to study are more influenced by nutrition (and perhaps body composition), whereas the similarities could be the consequence of uremia, with malnutrition playing a less important role.

Both plasma glycine and alanine concentrations showed a significant inverse relationship with the plasma urea, but no amino acid correlated with the plasma creatinine concentration. The efficacy of dialysis is difficult to measure. All the children were dialyzed similarly for 30 hr each week with membranes of appropriate size to body surface area and the plasma creatinine and urea are probably influenced more by the patients' muscle mass and protein intake, respectively. Notwithstanding this, it is possible that the changes in these two amino acids were independent of the efficacy of the dialysis procedure (as evidenced by the plasma creatinine), but that they may have been influenced by other factors which could elevate the urea, such as high protein intake or endogenous protein catabolism. While neither amino acid correlated significantly with the protein intake, Table 4 shows that the plasma glycine concentration tended to vary inversely with the protein intake.

In the present study overt clinical signs of malnutrition were not present, but growth retardation and delayed bone maturation frequently were. Dietary intakes recorded in our children and expressed according to actual body weight or height age seem adequate, but in the face of chronic malnutrition there may be greatly increased energy requirements for catch-up growth [18], which, given the other constraints of uremia, may be difficult to satisfy. When the dietary intakes are expressed in relation to chronological age they are inadequate. The dietary recording was not supervised and the mean coefficient of variation in five diet assessments per child was 15% (range, 6 to 28%) but despite this the plasma glycine still falls as energy intake rises, even when the mean of several plasma glycine estimations over a six-month period for each child is analyzed in relation to the mean of the intakes over the same period. In fact, the correlation between plasma glycine concentration and fat and energy intake then becomes significant at the 1% level and protein intake also becomes a significant factor [19]. We do not know which method of dietary assessment is most representative.

In contrast to our findings on valine and glycine, Kopple and Swendseid [5] have shown that the plasma valine/glycine ratio was affected both by dietary protein and by uremia, being most reduced in subjects on hemodialysis consuming a low-protein diet. It is not clear whether the ratio was altered by changes in glycine or valine concentration, or both, and whether the diet had any effect on the plasma valine concentration at all. Swendseid et al [20] have shown that the plasma valine concentration falls rapidly in young healthy adults when their protein intake is sharply reduced. We have found that in a group of uremic children, despite considerable variation in protein intake (0.5 to 4.8 g/kg of body wt/day), the plasma valine concentration did not show related variation, although it was reduced in the group as a whole. It may be that, taking each child as his own control, a further reduction in plasma valine concentration might occur if protein intake is sharply reduced, but the distinct lack of correlation of valine

with diet suggests that its alteration is due more to effects of the uremic state on protein metabolism. The plasma albumin concentration also did not correlate with diet and possibly its relation to plasma valine indicates that both reflect the nutritional state of the individual whether there is protein deficiency for metabolic or dietary reasons. The plasma glycine on the other hand may be more responsive to alterations in the actual dietary intake.

If it is accepted that the intakes of these children, while not fulfilling the requirements for much catch-up growth, are adequate according to body size, then the changes in the amino acid concentrations we have found may be metabolic in origin. Essential amino acid losses in the dialysate are not greater than nonessential amino acid losses and are easily replaced by the diet [17].

There is evidence that glucose [21] and lipid metabolism [22] are abnormal in uremia, with peripheral resistance to insulin and thus impaired utilization of glucose with hyperinsulinemia. Either secondarily or independently, lipid uptake appears to be reduced [23] and lipolysis may also be reduced [24]. These may interfere with the supply of energy substrate, and amino acids may be diverted from protein production to energy, a paradoxical finding in the face of hyperinsulinemia which stimulates the cellular uptake of amino acids. However, a similar metabolic state is seen in sepsis accompanied by acute starvation [25].

In chronic protein wasting states, as in uremia, the plasma concentrations of the branched-chain amino acids are reduced [26]. Preferential oxidation of the branched-chain amino acids to CO_2 occurs in muscle [27] and we suggest that with impaired utilization of conventional energy sources in uremia, oxidation of the branched-chain amino acids occurs to provide energy, leaving a surfeit of other nonessential amino acids. The raised plasma alanine concentrations in some patients may be an indication of its increased peripheral release as essential amino acids are used up for energy. Disordered lipid metabolism is in part shown by high concentrations of plasma triglycerides (TG) and we have found that as energy intakes increased TG concentrations tended to fall [19]. The mean plasma alanine concentration at the upper limit of normal disguises how several patients had considerably elevated concentrations (Fig. 2), which were associated with elevated TG concentrations. However, diet had no effect on alanine concentration and these abnormalities may be present for unrelated reasons, although it seems appropriate to try and explain them by a single hypothesis of altered energy metabolism.

The fall in plasma amino acid concentrations after glucose in the present study is similar to that described in healthy subjects [28]. It is insulin-controlled and does not occur in insulin-dependent diabetes [29]. The fall in alanine concentration, which we found, is not typically found in response to glucose, or insulin, where no change or even an increase may occur [28, 30, 31]. In three of the children its concentration did rise after glucose. We have found that those patients with the poorest glucose uptake had the highest fasting alanine concentrations, with the greatest reductions (to normal levels) after glucose. Possibly those patients with the poorest glucose tolerance were those who used up more essential amino acids for energy. When augmented insulin secretion in response to glucose occurred, there was a resurgence of protein synthesis with a reduction in the release of previously unusable nonessential amino acids, including alanine.

Raised citrulline concentrations in uremia appear to be a consequence of reduced extrahepatic utilization, as its production in the liver is actually reduced

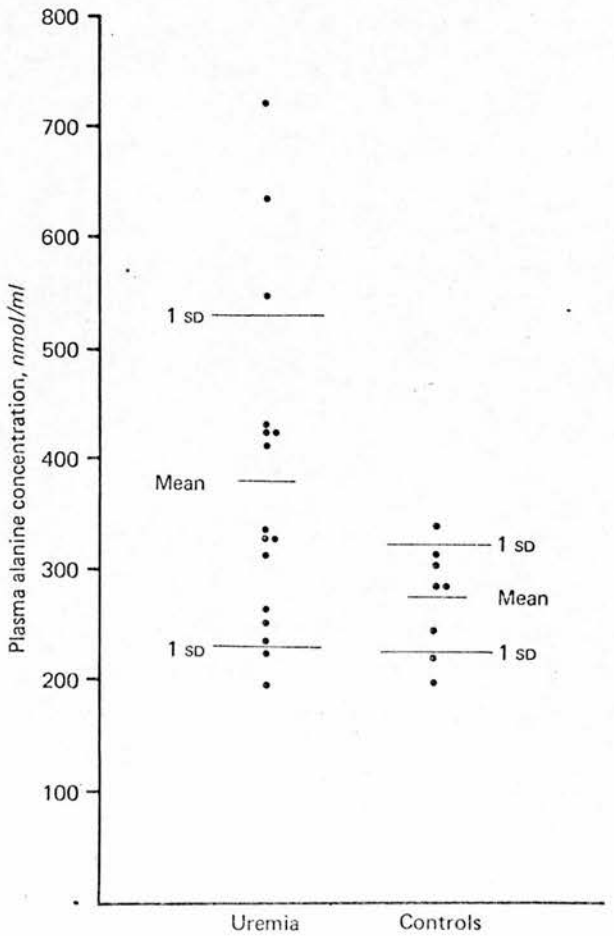

Fig. 2. *Plasma alanine concentrations in uremic and control children, nmol/ml.*

and subsequent intrahepatic metabolism normal in uremia [6]. Its direct relation to protein intake suggests that as intake improves either its production increases, or by some "toxic" effect its utilization is further reduced.

Certain correlations between some biochemical and other changes in uremia have been demonstrated in this study and we have suggested that some of these are causally related. It is true that our comparisons are of variations in a heterogenous group of patients and without controlled variation of a single parameter they must be interpreted cautiously. A more precise study is needed, therefore, to determine these individual aspects, and to determine whether a single hypothesis of disturbed energy metabolism is tenable.

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